A STUDY ON PHYTOCHEMICAL AND BIOLOGICAL ACTIVITY OF CARISSA CARANDAS Linn

A Thesis Submitted in Partial fulfillment of the Requirement for the award of Degree of

DOCTOR OF PHILOSOPHY

IN

PHARMACY

By

Mr. Mallikarjun Malipatil

Under the Supervision of

Prof. Ramesh Chandra

Former Vice-Chancellor, Bundelkhand University Jhansi

Founder Director, Dr B. R. Ambedkar Centre for Biomedical Research

Delhi University, Delhi

Submitted to

BUNDELKHAND UNIVERSITY, JHANSI

MAY 2007

Dedicated to my family

CERTIFICATE

This is to certify that thesis entitled 'A Study on Phytochemical and Biological activity of *Carissa Carandas* Linn' in partial fulfillment of the requirement for the award of degree of Doctor of Philosophy in Pharmacy, submitted to Bundelkhand University, Jhansi by Mr. Mallikarjun Malipatil is based on the results of experiments carried out by him under my supervision.

I hereby forward his thesis.

Date:
Place:

Co-Guide
Dr. Raghuveer IRchhaiya
Head & Reader
Institute of Pharmacy
Bundelkhand University, Jhansi

Rameshchandra
Prof. Ramesh Chandra
Founder Director
Dr. B. R. Ambedkar Centre for
Biomedical Research
Delhi University, Delhi

DECLARATION

I hereby declare that the research work entitled 'A Study on Phytochemical and Biological activity of *Carissa Carandas* Linn' has been carried out by me under the guidance of Prof. Ramesh Chandra, Former Vice-Chancellor, Bundelkhand University Jhansi and Founder Director, Dr. B. R. Ambedkar Centre for Biomedical Research Delhi University Delhi, is original in its contents and has not been submitted elsewhere for award of any other degree.

Date: 26/05/07
Place: Delhi

Mr. Mallikarjun Malipatil

DIRECTOR & HEAD
Institute of Pharmacy
Bundelkhand University, Jhansi

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Date: 26/05/07 ***Place:*** Delhi

Mr. Mallikarjun Malipatil

LIST OF ABBREVATIONS

C. carandas	*Carissa carandas*
PE	Petroleum Ether Extract
ME	Methanolic Extract
CE	Chloroform Extract
EAE	Ethyl acetate extract
BE	Benzene Extract
CCME	*Carissa carandas* methanolic extract
LC-MS	Liquid chromatography mass spectrum
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transfer Infrared
WHO	World health organisation
TLC	Thin Layer Chromatography
TS	Transverse section
MIC	Minimum Inhibitory Concentration
S.E.M.	Standard Error of Mean
DMSO	Dimethyl Suiphoxide

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INTRODUCTION

INTRODUCTION

Natural products (crude drugs, extracts and pure compounds) have been derived from higher plants, microbes or animals and these can be of either terrestrial or marine / aquatic origin. Over the last decade, interest in drugs of plant origin has been growing very fast and currently, there is world-wide upsurge in the use of herbal preparations and active ingredients of medicinal plants in health care. The consumption of medicinal plants has almost double in Western Europe in recent years. The ecological awareness and an increased demand for non-classical therapies may be invoked as the main reasons for the renewal. Equally, the efficacy of a number of phytopharmaceutical preparations, such as ginkgo, garlic or valerian, has been demonstrated by studies that applied the same scientific standards as for synthetic drugs. As a result of all reasons, there is an enormous market for crude herbal medicines in addition to purified plant derived drugs ((Hamburger and Hostettmann, 1991).

The medicinal preparations based on these raw materials were in the form of crude drug such dried herb, or an extract thereof, and are invariably derived from a mixture of several materials. With the advent of European scientific methods, many of these reputed medicinal plants came under chemical scrutiny leading to the isolation of active principles. Beginning with 1800 AD there was continuous activity in this area and many of the well known medicinal plants were chemically analyzed and their active principles characterized. Soon after their isolation and characterization, this compound, either in pure state or in the form of well-characterized extracts, became part of Pharmacopoeias of several countries. This is where Herbal Medicine and Modern Medicine have a common link (Farnsworth, 1980).

After centuries of empirical use of herbal preparations, the first isolation of Active principles especially alkaloids such as morphine, strychnine, quinine etc., in the early 19th

century marked a new era in the use of medicinal plants and the beginning of the modern medicinal plants research (Hamburger and Hostettmann, 1991;De Pasquale, 1984; Phillipson, 2001). Prior to world war 2, a series of natural products isolated from higher plants became clinical agents and a good number of them are still in use today e.g., quinine morphine, codeine, digoxin. The antibiotic era dawned during and after World War 2, due to the antibacterial effects of whole series of natural products isolated from species of Penicillum, Cephalosporium and Strptomyces, In The post-war years there were relatively few discoveries of new drugs from higher plants were done, e g., reserpine, vinblastine and vincristine which are still effective in cancer chemotherapy (Phillipson, 2001). Since 1949, another 2000 new medicinal herbs have been identified (Bannermann, 1982).

It is estimated that 60% of anti-tumor and anti-infectious drugs already in the market or under clinical trial are of natural origin and the vast majority of these molecules cannot yet be synthesized economically and are still obtained from wild or cultivated plants (Rates, 2001). The isolated compounds artemisinin, taxol and campothecin are examples of natural products that are undergoing clinical trial and commercial development. Several natural products isolated from plants used in traditional medicine have potent antiplasmodial action in vitro and represent potential sources of new anti-malarial drugs (Phillipson, 1994). In addition to above all a good number of novel plant derived substances have entered into western drug markets and clinical plant based research has made a rewarding progress in the important fields particularly in anticancer and anti-malarial therapies (De Smet, 1997).

Thus, many of existing drugs have derived directly or indirectly from higher plants and many medicinal plants are serving as sources of direct therapeutic agents, raw materials for the manufacture of more complex compounds, models for new synthetic

products and as taxonomic markers (Akerele, 1993). Several classic plants drugs have lost much ground to synthetic competitors and some others have gained a new investigator or therapeutical status in recent years (De Smet, 1997).

Carrisa carandas Linn is a large evergreen shrub with a short stem, glabrous except the inflorescence; bark light gray, scaly; branch lets usually alternate, with thin stout sharp horizontal glabrous spines 2.5-3.8cm.long at their base; branches usually with out spines. Leaves 3.8-7.5 by 2.5-5.0cm, coriaceous, ellepatic or obovate, obtuse, often shortly mucronate, glabrous and shining, base subacute; petioles 3.6mm long. Flowers white, scarcely odorous, in pubescent terminal corymbose cymes; peduncles usually 6.0-20mm long, sometimes almost zero. Pedicels very short; bracts linear, pubescent. Calyx pubescent, cleft rather more then ½ way down; lobes 2.5-3.0mm long, lanceolate, vary acute, celiate. Corolla tube about 1.3 cm long swollen and pubescent near the top; lobes 1cmlong, oblong - lanceolate, acute and pubescent. Stamens included within the corolla tube; filaments vary short; anthers linear-oblong. Ovary glabrous; ovules 4 in each cell; stigma slightly pencillate at the apex. Fruit 1.3-2.5cm long, ellipsoid, purplish black when ripe, moth 4 0r more seeded (The wealth of India, 1992).

The first chapter of the thesis explores the background, need for the study; present international and Indian status, hypothesis and current strategies. In this chapter a number of different systems of herbal medicine from around the world and throughout history has been discussed in brief. An effort has been made to briefly review and explore the philosophical and therapeutic importance of the traditional Indian medicine, Ayurveda and folk and native medicines. The phytochemicals explored from these systems are discussed in brief. The plant has been described with respect to its pharmacognostic nature, phytochemicals and medicinal applications. All this is integrated into identified common themes and even viewed from the perspective of standardization,

characterization and pharmacological evaluation. The objectives of the present study are discussed in detail and in general in the chapter II.

A useful review of pharmacognosy, phytochemistry and herbal pharmacology follows in chapter III, discussing the important group of constituents responsible for antidiarrheal, anti-microbial, adaptogens, antistress, antidiarrheal and antiulcer properties. Other important medicinal plants along with *Carissa carandas* and their constituents are also revised in detail with respect to their pharmacological properties considered in the present study. The review of literature has indicated that till the date no detailed pharmacognostic, phytochemical and pharmacological activities are studied in detail.

Use of an ideal and authenticated herbal medicine is an essential part of an effective herbal therapy. The standardization of herbal preparation remains as a biggest challenge and need of the hour to avoid adulteration, toxicity and lack of potency. In chapter IV *Carissa carandas* roots, pharmacognostic and standardization methods including morphology and microscopic properties are depicted along with results. The total ash value, acid insoluble ash value, water and alcoholic soluble extractive values and moisture content evaluation is given in detail for able to reproduce and utility for standardization.

Principles of herbal treatment are articulated with the emphasis one would expect from phytotherapists so well grounded in holistic medicine. These phytochemicals provide clear acknowledgement that competent phytotherapy is more than simply the use of herbs. Modern system of medicine is more focused on use of single active constituent rather than use of crude extract due to their reproducibility and ability to reproduce by semi synthetic and synthetic process. To evaluate the active constituents of *Carissa carandas* root, it was extracted by successive solvent extraction, extract has undergone fractionation to isolate, purify and identify the active constituents the phytochemical

study was carried out. The detailed methodology, results, spectra and its interpretation are given in chapter V.

An invaluable section of the thesis chapter VI is an in-depth *materia medica,* includes the scientific validation and pharmacological evaluation of *Carissa carandas* root preparation. In this section research methodologies, pharmacological properties, acute toxicity and other limitations and perspectives of developing an effective phytotherapeutic are explored. A discussion of safety, efficacy, adverse reactions, cytoprotective and anti-infective properties along with blind screening based central activities are evaluated and described in relation with previous studies. The broad issues investigated are infectious diarrhea, infectious ulcer, central disorders, biliousness, and traditionally claimed adaptogenic properties. Each activity is presented in a well-structured, fully referenced way, blending traditional views with insights from phytochemistry, pharmacognosy and pharmacology results of present investigation.

In brief, but significantly outcome of the present study is given in chapter VII as summary and conclusion. It strands as an invaluable, extensive, well-constructed index for others before beginning the study on *Carissa carandas* root preparation. It also summarizes outcome of the present investigation with concrete decision and directions for further research and investigation with possible and probable outcome.

OBJECTIVES

OBJECTIVES

New plant drug development programs are traditionally undertaken by either random screening or an ethanobotanical approach, a method based on the historical medicinal / food use of the plant. One reason there has been resurgence in this area is that conservationists especially in U. S. A. have argued that by finding new drug leads form the rainforest, the value of the rainforest to society is proven. Presently, there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases. Numerous drugs of Chinese medicine have entered the international market successfully through exploration of ethnopharmacology and traditional medicine. Although scientific studies have been done on a large number of Indian botanicals by using crude plant preparations leading to a considerably smaller number of marketable drugs or phytochemical entities entering the evidence based therapeutics. Efforts are therefore needed to standardize, isolate the active ingredient, establish and validate evidence regarding efficacy, safety and toxicity of traditional herbal medicines of Indian origin.

Bioassay-guided study of natural products will leads to an important and effective medicinally useful active constituent with a possibility of reproducibility and clinical applications as an early lead. The *Carissa carandas* has been studied for its pharmacognostic property, phytochemistry and pharmacological properties way back in 1960's and 70's but no further studies have been carried out till the date except its antiviral property in last decade. However, it finds a significant place in the Ayurvedic Pharmacopeia of India and has also been described in some monographs, but none have described the complete pharmacognosy, chemistry and pharmacology of this important ethnomedicinal plant. Therefore, we aimed to investigate its pharmacognostic properties

to enable its standardization, isolate and identify active constituents and find out its pharmacological applications. In the present study an effort has been made to standardize, explore, separate, isolate, identify along with their pharmacological evaluation of some new compounds with a desire to obtain highly potent, more specific, and less toxic drugs.

The detailed objectives of the present study are as follows,

1. To study morphology and microscopy of thin and thick root of *Carissa carandas.*
2. Standardization of *Carissa carandas* roots by physico-chemical parameters including , total ash value, acid insoluble ash value, alcohol soluble extractive value, water-soluble extractive value and moisture content.
3. To extract by successive solvent extraction, extract fractionation, isolation, and identification by FTIR, ^{1}H NMR, and LC-MS spectral studies.
4. Pharmacological evaluation of extracts for anti-microbial, antiulcer, antidiarrheal, adaptogenic and antistress activity based on its traditional claims.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Diseases are born with man and drugs came into existence since a very early time to remove the pain of diseases and cure them. Thus, the story and history of drugs old as the human civilization. Drugs used in medicine today are either obtained from *nature or* of *synthetic origin.* Natural drugs obtained from plants, animals, or mineral kingdom. Drugs from microorganisms like antibiotic were not known in the early period. Synthetic drugs like aspirin, sulfa drugs, some vitamins, and some antibiotics are synthesized in the laboratory from simple chemicals through various chemical reactions. Little work was carried out by the pharmaceutical industry during 1950-1980; however, during the 1980 – 1990,s massive growth has occurred. This has resulted in new developments in the area combinatorial chemistry, new advances in the analysis and assaying of plant material and a heightened awareness of the potential plant materials as drug leads by conservationists. However, tropical forests have produced only 47 major pharmaceutical drugs of world – wide importance .It is estimated that a lot more, say about 300 potential drugs of major importance may need to be discovered. These new drugs would be worth $ 147 billion. IT is thought that 1, 25,000 flowering plant species are of pharmacological relevance in the tropical forests. It takes 50, 000 to one million screening tests to discover one profitable drug. Even in developed countries there is a huge potential for the development of neutraceuticals and pharmaceuticals from herbal materials. For example UK herbal materia medica contains around 300 species; whereas the Chinese herbal material medica contains around 7000 species .One can imagine what lies in store in the flora-rich India.

Historical aspects of use of medicinal plants:

The disease, death and decay are co-existing with life. The art of using medicinal plants, as a source for relief from illness and to cure diseases is as old as mankind. In the earliest times, the medicinal plants were crucial for sustaining the health and well being of the mankind. The medicinal plants were not only being used as curative agents but also used for life enhancing benefits (Hamburger and Hostettmann, 1991;Chevallier, 2001; De Pasquale, 1984; Phillipson, 2001). The Primitive man has found it necessary to defined his physical integrity and to cure his wounds and other illness with the help of instinct, luck and also through observation. Hence, even today in many traditional societies-the treatment is interceded with the spiritual realm to bring about a cure (Chevallier, 2001; De Pasquale, 1984).

It is very difficult to tell about the exact beginning and historical development of the use of plant use of plant source for therapeutic purpose. But, it is believed that the use of plant source for therapeutic purpose begun about 60 thousand (d) years ago, when Neanderthal man begun using the same. It is also believed that, the Neanderthal man possessed a rudimentary pharmacopoeia. The written documents for the use of plant source for therapeutic purpose are available since from five millennia, the time when early civilization of china, India and North Africa started recording the observations they made (Hamburger and Hostettmann, 1991; Chevallier, 2001). During 7^{th} century B. C., people of Mesopotamia were used to have many Ayurvedics in the form of tablets called the cuneiform drugs. During the 4^{th} and 3^{rd} century B.C., Greeks were very famous in the medicinal field and they started the cultivation of medicinal plants and extraction of oils from them. As early as 3^{rd} millennium B.C., there had been exchanges between Egypt and India either means of the caravans that followed the trails running parallel to the Elbuz Mountains in the North of Iran and from their south through Beluchistan or else by means

of ships that communicate through the Persian Gulf from the Indo to the Tigris. The most important document that gives complete information of history of Egypt medicine named *"Materia medica"* was discovered in 1873 and was written during 3300-2600 B. C. This is considered as one of the most ancient Pharmacopoeias and principal source of information of Egyptian medicine, which report numerous medicaments of the mineral and animal origin besides a considerable number of vegetable drugs and many of which are still in use. The another document Veda (book of knowledge) written around 2000 B. C., in particular, the Athurveda contains all the medicinal references for the cure of many maladies during that time. During 1990 B. C., pharmacists roamed about the villages selling spices, gums, balsams and different medicines in Egypt and Israel. The evidences those are available from the paintings of the valley of the queens in Luxor (Egypt) tell that the Queen Hatshepsut (1504-1483) sent an expedition, in which some doctors took part to Africa from where various drugs were brought back. Most of which analyzed recently and found to have many important drug constituents. The Chinese Emperor Chen Nong, who lived about 2700 years B.C., around Chou period (1122-221B.C) honored as god of medicine and written a Chinese famous and oldest *Materia Medica* called Chen *Nong Pent 'ts' ao*. A version of this book, the *Pent ' t, s ao kang Mu* considered as a real Pharmacopoeia, which is having records for the treatment of many chronic diseases. During Incas period (2000 years back) quacks named as itinerant apothecaries roamed about over the whole region with their stock of simple remedies for diarrhea, eczemas, scabies and eye infections and also as purgative and abortifacients (De Pasquale, 1984). In Europe during 1st century A.D., the Greek Physician of Roman army named Dioscorides was very famous and he traveled in Egypt, Africa, Spain and Italy and collected the information regarding the medicaments known by that time. He wrote first European book on herbal medicines known as *De Materia Medica*.He Listed about 600

herbs of medicinal applications and they were to have an astonishing influence on Western medicine. This book was being the principal reference used in Europe until the 17th century (De Pasquale, 1984; Chevallier, 2001). Galen, the physician to the Roman emperor Marcus Aurelius had an equally profound influence on the development of herbal medicine by drawing inspiration from Hippocrates. On the other side of the world, the ancient Mayan, Aztec and Inca civilizations all had a profound understanding of local medicinal plants. At the same time, medicine and religion were still closely interwoven possibly, even more so than in Europe. In illness, herbal medicine supplemented a supernatural appeal to the Gods (Chevillier, 2001).The old Mexican civilizations more than all other cultures, possessed the secret of drugs which produce hallucinations, analgesia, narcosis, physical well being agents, anesthesia that lasted for several hours and agents which stimulate sexual potency. They were also having the idea to prepare the tonic beverage using the cacao bean, vanilla, honey and pepper. Besides the tobacco was being used as a remedy for headaches, giddiness and in diseases of the primary respiratory organs, the gums and juice of the tree was also used for plasters (De Pasquale, 1984). Western medicine suffered with the decline of the Roman Empire. All the Documents were lost but the Arabic culture that was gain of the classical Greek and Roman period were preserved and elaborated. The Arabs were expert pharmacists and their contacts with both Indian and Chinese medicinal traditions meant that they had a remarkable range of medicinal and herbal knowledge. Further east, in India, the 7thAD, century saw a golden age of medicine with scholars recording the achievements of the time, which saw the blossoming of hospitals, maternity homes and medicinal herb gardens (Chevalier 2001). During 8th century AD, after the fall of Roman Empire, Arab and Indian medicinal system became famous. About 200 years ago, Chinese produced

18th volume of textbooks of medicine containing sections on nutrition, diet and hygiene, and over 100 medical prescriptions (Bannerman, 1982).

Importance of medicinal plants:

Since 60 thousand years, humans have relied almost entirely on plants to treat all manners of illness; from minor problems to life-threaten diseases (Chevallier, 2001). About 80% of the world's people still depend largely on traditional plant derived drugs for their primary health care (Akerele, 1993). In the developing countries, the herbal medicine is deep rooted even today. This is because of, (a) the traditional medicine is still practiced to some degree in all cultures, (b) traditional medicine is only source of health for many people and for them " primary health care" is synonymous with traditional medicine and (c) traditional practitioners play important role in their communities particularly in regard to common ailments and chronic disorders (Bannermann, 1982). The discovery of the penicillin from microbes and isolation of antimalarial alkaloids, cardiotonic glycosides, anticancer agents, etc., the plants are the significant evidence for importance of the natural products for the benefit of mankind.

Despite the belief that the majority of clinical drugs are synthetic in origin, it is interesting to note that 6 out of the top 20 pharmaceutical prescription drugs dispensed in 1996 were natural products and that over 50% of the top 20 drugs could be linked to natural products research (Phillipson., 2001). It is estimated that in 1997 the world market for over-the-counter phytomedicinal products was US$ 10 billion, with annual growth of 6.5%. Presently, about 25% of the drug prescribed worldwide comes from plants and 121 active compounds from natural products are being in current use. Of the 252 drugs considered as basic and essential by the WHO, 11% are exclusively of plant origin and a significant number of synthetic drugs obtained from natural precursors (Rates, 2001). There are 119 drugs of known structure that are still extracted from higher plants and

used globally in allopathic medicine. These 119 plants derived drugs are produced commercially from less than 90 species of higher plants (Farnsworth, 1990).

In addition to above mentioned all facts, the extensive practice of traditional medicine in developing countries and the rapidly growing demand for the alternative and basic therapeutic means in industrialized countries constitute the international relevancy of research and development in the field of traditional herbal drugs. An additional motivation for such activities is found in the practical necessity to integrate the potential of traditional herbal medicine into current practices of modern health cares (Labadie, 1986).

Scope for medicinal plants:

Emphasis shifted away from plant-derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation soon after 1945. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint during this period (Hamburger and Hostettmann, 1991). But, further during later part of the 20th century herbalism has become mainstream world-wide, this is due to several reasons, the significant once are ; (a) the value of traditional and indigenous pharmacopoeias ,which make health care affordable for all,(b) conventional medicine can be inefficient (e.g. side effects and ineffective therapy),(c) abusive and/or incorrect use of synthetic drugs result in side effects and other problems (d) large percentage of world's population does not have access to conventional pharmacological treatment, (e) folk medicine and ecological awareness evidences that the natural products are less harmful, (f) herbal medicine often complements conventional treatments and (g) provides safe, well-tolerated remedies for chronic illness (Rates, 2001; chevalier, 2001).

The clinical applications of taxol, etoposide and artemisinin have helped to revive an interest in higher plants as sources of new drugs. Though there is a considerable development in medicinal field there still remains an urgent need to develop new clinical drugs for numerous disease, which result from the malfunction of the central nervous system (CNS),e g. Alzheimer's and Parkinson's disease, epilepsy, migraine, pain, schizophrenia, sleeping disorders, etc. Natural products have already proven an track record for CNS activities, e.g. caffeine, codeine, morphine, nicotine, reserpine and it is possible that there are further such drugs still to be found from nature (Phillipson, 2001). The search for the macrofilaricidal drug in indigenous plants is continued due to non-availability of the drug for the same purpose (Comely, 1990) during resent years, the attention of the pharmaceutical industry has switched once more to natural world and this may be illustrated by the reference to three clinical drugs, taxol, etoposide and artemisinin, they are giving much importance to natural product research especially herbal products to find the lead molecules for various disease, besides these industrial research, many institutes and Universities are also concentrating much on herbal product research e.g., the NCI (National cancer Institute, USA) has tested more than 50,000 plant samples for anti-HIV activity and 33,000 samples for anti-tumor activity (Rates, 2001). Tremendous nature products work has been done during 1940s-1970s, despite these discoveries the impact of the phytochemistry on new drug development waned inevitably the innovative pharmaceutical industries turned to synthetic chemicals (Borris, 1996; Phillipson, 2001).

Large pharmaceutical companies, such as merk, CIBA, Glaxo, Boehringer and Syntex, now have specific departments dedicated to the study of new drugs from natural sources (Rates, 2001). Glaxo Company is well known for the production of drugs in bulk which

are identified as drugs from natural products e.g. penicillin, vitamin B_{12}, etc., and it is giving much importance to further natural product research (Turner, 1996).

The WHO considers phytotherapy in its health programs and suggests basic procedure for the validation of drugs from plant origin in developing countries. Eastern countries, such as China and India have well established herbal medicines industries and Latin American countries have been investing in research programs in medicinal plants and the standardization and regulation of phytomedicinal products following European countries such as France and Germany (Rates, 2001). WHO has hosted interregional workshops to address methodologies fro the selection and use of traditional medicines in national "Primary health care" programs (Akerele, 1993).

However, the potential use of higher plants a source of new drugs is still poorly explored. Of the estimated 250,000-500,000 plant species, only a small percentage has been investigated pytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties. In most cases, only pharmacological screening of preliminary studies has been carried out and it is also estimated that only 5000 spices has been studied for medicine use (Rates, 2001; Hamburger and Hostettmann, 1991). It is logical to presume that many more useful drugs will be found in the plant kingdom if the search for these entities is carried out in a logical and systematic manner (Farnsworth, 1990). Also theories of Galenic, Ayurvedic (Indian), and Chinese medicine, however, would have meant little too most of the world's population, which is still relied on the services of local "wise men and women" (Chevallier, 2001).

Plants used/ being used for birth control:

Plants have been used in traditional medicine for many centuries as abortifacients, contraceptives, lactogenics, aphrodisiacs, for menstrual regulation and for fertility control (Bannerman, 1982). During 1st century AD itself, Dioscorides distinguished the

differences among contraceptives, menstruation provokers, and abortifacients in his *De materia medica.* He further classified plants for drying out the menses, purging the after birth, killing an embryo and aborting the conceptus. Dioscorides and the physician Soranus agreed on the powers of such emmenagogues and abortifacients and antifertility effect of many plants those were known during that period (Meyer and Eldrege, 1998). These are the evidences that during Aztec times (Azitec medicine) medicinal plants were being used for the contraception, abortion and to cure many sexual related abnormalities (quazda, 1975). Scholarly Bishop Macer's 11th Century listed menstrual regulators, abortifacients and contraceptives in *De virtutibus Herbarum,* written by him about the use of herbal medicine. The German physician Canonized as saint Hilgegerade of Bingen enumerated seven plants as emmenagogues or abortifacients including tancy, oleaster and nasturtium for a woman not menstruating for "so long a time that it hurts" in *De simplicis medicinae*. Another 12th Century woman physician and medical writer, Dr. Trotula of Salerno, recommended Artemisia wine as an abortifacient, if that fails, a potent drink combining hemlock,castorene, artemisia, myrrh, century plant and sage. Ancient law and literature preserve abundant references to abortion and contraception in the writings of Plautus, Juvenal, Ovid, Philio, Lucretius, and Seneca, as well as early Christian writers as Clement of Alexandria, john Chrysostam, Lactantius, Ambrose, and Jerome. Classical court cases and legislation restricting abortion never refer to a fetus's "rights" but rather the rights of the parent deprived of an heir (Meyer and Elderege, 1998).

The Indians, who were not treated well by their Dutch masters, used the seeds of plant *caeselpinia pulcherrima* (L.) to abort their fetuses, so that they will not become slaves like themselves. The black slaves from Guinea and Angola have demanded to be well treated threatening to refuse to have children by the use of available herbal contraceptives (Schiebinger, 2000). Over 1200 medicinal plants that were commonly

used/ being used in folk medicine for abortion in eastern Africa is listed by J.D Kokwaro (Yu, 1982).

The large number of plants, which were traditionally used and are being used in South America to control the fertility and were tested for their contraceptive effectiveness in different animal models (Azorero and Schavartzman, 1975; Colmeiro-Lafforet, 1962). Popular medieval antifertility plants such as artemisia, myrrh, pennyroyal, rue, squirting cucumber and especially, Queen Anne's lace endure today as folk abortives. In mice, rats, guinea pigs, and rabbits, Queen Anne's lace plant seeds apparently prevent ovum implantation, inhibit ovarian growth, and disrupt the estrus cycle (Meyer and Elderege, 1998).

Now, we can explain chemical effectiveness that was only empirically understood earlier. When tested in modern labs, some historical substances disrupt or desynchronize preovulatory and preimplantation events in animal and human beings. The various components present in herbal medicines, which exert antifertility effects are isoflavones or uterine contractors or estrogenic steroids that act on the hypothalamus and pituitary gland or prostaglandin stimulants inhibiting sperm transport or uterine implantation. Since antiquity, people have known how to upset the delicate hormonal balance necessary to postovulatory reproduction (Meyer and Eldrege, 1998).

The isolation of lead antifertility molecule from medicinal plants was started in 1952 with the isolation of a simple aromatic compound, 2, 6- dimethylhydroquinone from the common garden pea. Afterwards, a much attention was paid towards the natural product chemistry to isolate the non-steroidal antifertility agents from indigenous plants in all over the world. As a result of screening of much number of their antifertility effect in different animal models and their activity fractions were taken for further isolation work. The compounds Gossypol [1, 1', 6, 6', 7, 7'- hexahydroxy-5, 5'-disopropyl-3, 3'-

dimethyl-(2,2'-binaphthalene)-8,8'-dicarboxaldehyde],desmethylsoencecalin and 11hydroxyvincadifformine from plant genus *Gossypium, Blepharispermum* and *melodinous,* respectively, are exemplified here. Many of the isolated compounds are presently in clinical trial (Ciereszko and Dabrowski, 2000; Agarwal et al., 1999; Guo and Zhou, 1993). The recent study of Women's Health center in Geneva, Switzerland indicate that the herbs mentioned for contraception in the book "Natural healing in Gynecology: A manual for women" was evaluated and they are 60-80% effective to control fertility in women with minimal side effects (http://www.orgonelab.org/contracep.htm). Many Indian institutes and Universities were /are taken the matter of search of new contraceptives in natural products as a serious talent, e.g., CDRI has contributed much by searching the fertility control substances www.cdriindia.org/fertility_control.htm). Thus the modern social context and economic view of health services, the needs of the pharmaceutical market and recognition that research on medicinal plants used in folk medicines represents a suitable approach for the development of new drugs (Rates, 2001). This research have led to an increase in the number of publications in this field of contraception and many private and governmental institutions are still financially supporting research programs world-wide

Utilization of Phytopharmaca :

The direct utilization of plant material is a feature of the systems of traditional medicine not only in the developing world but also in Europe where one finds a considerable resurgence of faith in plant-derived medicines. Preparation of decoction, tinctures, galenicals and total extracts also form a part of many pharmacopoeias of the world. The use of isolated pure phytoconstituents in medicine has been fully documented. For making synthetic drugs, the use of intermediates or starting

materials may be based upon plant products like diosgenin, hecogenin, and stigma sterol etc (Farnsworth,1985).

Poppy or Papaver Bracteatum:

Legal production of opium in India is to the tune of 2000 tons p.a., and this is primarily utilized for the production of most powerful pain killer morphine, cough suppressant drug codeine, and papaverine. But an illicit trade in opium has created an alarming situation in the production of street drugs, and resorting to the cultivation of another plant Papaver bracteatum total can prevent this drug abuse alkaloids of which contain 90% of thebaine (Farnsworth,1985).

Tropane Alkaloids:

These are used as parasympatholytics or as parasympathetic depressants and as cholinergic blocking agents. They are used in a variety of conditions to decrease secretion, relax smooth muscles and dilate pupils etc. Although Egyptian Henbane, i.e., Hyoscyamus muticus has been introduced in India with a view to have commercially viable yield of the two modern drugs-atropine and hyoscine, due to greater sensitivity of this plant to various diseases, it has not yielded fruitful results from commercial point of view. Further raw material for producing these alkaloids to meet not only national requirement but also to generate export potential will physochlaina, prealta which contains 0.88-1.2% of the total alkaloids containing more than 80% of hyocyamine. The plant occurs wild in dry inner valleys of Ladakh and Himachal Pradesh at altitude of 3500-4500 m. Among potentially rich areas are Kargil and Danther in HP., and Leh in Ladakh. Over exploitation of this plant needs to be avoided and plantations is needed for the future (Farnsworth,1985).

Dioscorea/Costus for Diosgenin:

World demand of steroidal drugs is going to be doubled by the end of the century. Factors governing steroid industry in India primarily include availability of raw material which is commercially obtained from Dioscorea. Fortunately, high yielding varieties D. floribunda and D. composite grow well in our country. India produces 25-30 tons of diosgenin at present but China is producing 300 tons. The world demand is 2200-2300 tons. In order to promote export we may behave to resort to other plants as well, like Costus speciosus (Zingiberaceae). The total biomass of its tuber is much more than dioscorea. Costus plant occurs as a weed in orchards and on the boundaries of cultivated fields of Arunachal Pradesh, Nagaland, Meghalaya and in the tropical rain forests of Tamil Nadu and Kerala. The plant can be easily cultivated in plains (Gbile,1987).

Pitfalls in Plant Drug Research:

The major pitfalls and stumbling blocks encountered in medicinal plant research all over the world continue to baffle and harass the Indian scientists too. Important among these factors are lack of standardization, nomenclature (many plants under one name, one plant having many names), botanical identification dosage formulation and frustrating active principles (Handa S.S, 1985).

Research Priorities :

In order to achieve results within a reasonable short period, it would be desirable to have "need based" approach to research on plant drugs. Research efforts could thus be directed in finding remedies for the so called "refractory diseases", i.e., those for which modern medicine has not been able to offer, so far, a satisfactory or lasting remedy. Toxicity of indigenous drugs is another area, which has been largely neglected.

Anti Cancer Drugs from Plants:

Cancer is an insidious disease affecting mankind in every country. Work on Periwinkle plant, Catharanthus roseus (L) G. Don, was independently taken up in two different laboratories for its alleged hypoglycemic activity as per Jamaican folklore. Though none of the groups could substantiate hypoglycemic activity, the Canadian group of Nobel Beer and cutts succeeded is isolating vinblastine while Eli Lilly group headed by Suoboda could isolated vinblastine and vincristine along with two other active dimeric alkaloids. These alkaloids are present in exceedingly low concentrations in a complex mixture of 50 alkaloids. Vinblastine was introduced (Velban, Eli Lilly) in 1961 and vincristine (Oncovin, Eli Lilly) in 1963 as anticancer drugs.

Screening of plant extracts for anticancer activity started in 1961 by National Cancer Institute of the U.S.A., and up to 1981 (20 years) about 1,41,045 plants had been screened, of which only 3.4% (representing about 3,400 different species) have been observed to be active in one or more bioassay systems. The promising phytoconstitutents, which are likely candidates for drug development, include indicine N-oxide (a pyrrolizidine alkaloid) from Heliotropium indicum, elipticine (a monomeric indole alkaloid) from several Ochrosia species, taxol from Taxus species and camptothecine (quinoline alkaloid) from Camptotheca acuminta (Chinese tree) (Handa S.S, 1971).

Plant drugs for Cardiovascular Diseases:

More than two-thirds of deaths results from heart attacks and it is number one killer in the world. In the area of cardiovascular drugs, plants are still the basis of treatment. Some of the plants accepted by most pharmacopoeias of the world include Digitalis, Convallaria, Adonis, Helleborus, Cratagus, which primarily act on the heart,

and Hydrastis, Veratrum, Ammi visnaga and Viscum album that act more specifically on the blood vessels. Phytoconstituents like digoxin, digitoxin, lanatoside from Digitalis species, K-strophanthidin from Strophanthus seeds, ouabain from Acokanthera schimperi, thevetin from Thevetia nerrifolia, Convallotoxin from Convallaria majalis, Scillaren from Urgenia scilla are commercially available cardiotonic pharmaceuticals. Hoechst is developing Colenol isolated from an Indian Labiatae plant Coleus forskohlii by CDRI under the name of FORSKOLIN as a hypotensive drug. Many more Indian plants are likely to give such leads in the future (Handa S.S, 1989).

Plants for Bronchial Asthma:

For bronchial asthma, lobeline from Lobelia inflate and ephedrine from Chinese plant Ma Huang (Ephedra species used for 5000 years in China) were introduced in medicine in 1925 by Chen of Eli Lilly and Co., U.S.A. This takes us to an example of development of a totally synthetic drug based upon a natural product model. An infusion of the leaves of Ammi visnaga has been used in Egypt and neighboring countries as a folk remedy for cough and colic. Its active principle was shown (1938) to be the furochromone khellin, which proved (1947) to be a bronchodilator. However, khellin was too toxic and several initial investigations aimed at improving its activity proved futile. Further investigations by another group finally led to the bis derivative, disodium chromoglycate which has since been marketed as an antiasthmatic drug under the trade name INTAL (Fisons) first in France (1969) and later in the U.S.A. (1973) (Handa S.S, 1984)..

Hepatoprotective plants :

A global estimate indicates that there are about 18,000 deaths every year because of the liver cirrhosis mainly caused by hepatitis. Hepatocelular caricinoma is one of ten most common tumours in the world with over 2,50,000 new cases each year. Although viruses are the main cause of liver diseases, the liver lesions arising from, xenobiotics excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon. Modern drugs have very little to offer for alleviation of hepatic ailments whereas most important representatives are of phytoconstituents used for liver diseases, chiefly on regional basis, include drugs like silymarin (Silybum marianum), catechin (Anacardium occidentalis and other) in Europe, glycyrrhizin (Glucyrrhiza glabra) in Japan and Schizandrins (Schizandra chinensis) in China. In India we have over 40 polyherbal commercial formulations reputed to have hepatoprotective action. Andrographis paniculata, and hepatoprotective activity has been established due to the presence of andrographolide. Kutkoside from Picrorrhiza kurroa is a potential hepatoprotectant reported by CDRI. Phyllanthus amarus is another most important plant selected for clinical trials (Handa S.S 1989).

Anti-inflammatory Herbal Drugs:

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest known diseases of mankind and affects a large population of the world, no substantial progress has been made in achieving a permanent cure. The greatest disadvantage in the presently available potent synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation. Therefore, the search of screening and development of drugs for their anti-inflammatory activity is an unending problem. There is much hope of finding antirheumatic drug from indigenous plants (Handa S.S, 1983).

Plants as Anti-diabetics:

As a present the total number of patients suffering from diabetes in our country is estimated to be 15.2 million. An epoch making discovery in 1921, that a pancreatic extract (insulin) would lessen the symptoms of diabetes, was a landmark in the area of drugs from natural sources. During World War II when insulin was not available in many countries, search was made for an insulin substitute from plant sources, Momordica chrirantia has been reported to be responsible for hypoglycemic activity (Handa S.S, 1983).

Plants for Urinary Stones :

The very first mention of 'Pashanbhed' in Ayurvedic literature is in Charka Samhita and is recommended for painful micturition and for breaking calculi. Sushrut Samhita mentions the drug under various synonyms-Pashanbhed for uric acid calculi and Ashnibhed for biliary calculi. Sushrut Samhita mentions decoction of Pashanbhed, Astimantaka, Satavari, Vrihati, Bhalluka, Varuna (Creataeva nervula), Kultha (Dolichos biflorus), Kola and Katak seeds for patients of Vataja Ashmari while Kusa, Ashmabhed, Patala, Trikantaka, Sirisha, Punarnava (Boerhaavia diffusa and shilajit are for Pittaja Ashmari. CRYSTONE of Himalaya Drug Co., and CALCURY of Charka Pharmaceuticals are already in the market(Handa S.S 1968).β

Sedatives/Tranquillizers :

Indian valerian (Valeriana wallichii) contains 2% valepotriates and is thus four times more potent than European valerian (V. Officinalis), which contains 0.5% valepotriates. These are trimesters of polyphydroxy cyclopenthanopyran esterified with isovaleric, acetic and ß-acetoxy valeric acid. Compared to known irriodoids, valtrats (valepotriates) are neither glycosides nor lactones and are considered original or primary products present in the drug responsible for activity. Valtrats are used as

tranquillizers and sedatives, and the action is comparable to meprobamate. Additional advantage is that these can be prescribed to alcoholic patients. Valerian also contains valerenic acid having spasmolytic action. Valeranone is found in Jatamansi (Nardostachys jatamansi) possessing sedative property. It will be worthwhile to produce valepotriates in India19.

Plants as Sources of Antimalarial Drugs:

It is estimated that there are about 215 million people chronically affected by malaria and that each year there are some 150 million new cases. Most hard hit areas include Africa, India Chine and East Asia.. A combination of resistance of Plasmodium species to antimalarial drug such as chloroquine, and the resistance of the vector mosquitoes to insecticides has resulted in malaria emerging as the world's most common tropical disease. A number of natural products have antimalarial activity and the best known example is quinine, the alkaloid which is derived from the bark of Cinchona species. Quinine has been used for many years as an antimalarial drug, even though at one time it was expected that its use would be eclipsed by the advent of a range of synthetic antimalarial drugs. In recent years clinicians have continued to use quinine because the resistance of Plasmodium strain is low when compared with other drugs.

Artemisinin is sesquiterpene lactones containing an endoperoxide moiety, which is a rare feature in natural products. Problems of flow solubility in both organic and aqueous solvents have been overcome by the development of sodium dihydroartemisinin hydrogen succinate monoester. Over 2,000 malarial patients have been treated with artemisinin in China. The Chinese use a number of other plants in traditional medicine for the treatment of malarial, including dichrea febrifuga (Saxifragaceae). Febrifugine, the active ingredient, has been used clinically against P.

vivax & P. ovale and even though it causes liver damage, it appears to be still in use as antimalarial in Chinese traditional medicine.

Simaroubaceous plants are used as antimalarials in widely different parts of the world including Thailand (Brucea javanica), U.S.A. (Castela nicholsoni), British Honduras (Picrasma antidesma), Mexico (Simaba cedron) and India (Ailanthus excelsa).

The active principles are thought to be a group of bitter substances quassinoids (simaroubolides) on which our laboratory has worked extensively, Bruceantin proved to be the most potent compound. The quassinoids without ester functions have little activity whereas those with ester functions at either C-15 or C-6 possess activity. Furthermore, activity has been demonstrated for quassinoids, which possess the oxygen bridge for C-20 to C-11 (glaucarubinone) as well as for those compounds with the oxygen bridge for C-20 to C-13, e.g., bruceantin (Handa S.S 1990).

Plants as Sources of Amoebicidal Drugs:

In our country there are 60 million cases today suffering from amoebic dysentery. There is no drug that can be considered ideal for the treatment of amoebiasis, particularly for the treatment of severe infections and although there are no reports of drug resistance at present but such a possibility cannot be ignored. Metronidazole is often the drug of choice for the treatment of most forms of amoebic dysentery, although, it does posses drawbacks in its lack of tolerance to alcohol and bad taste in mouth. There have been some reports of tumor formation in experimental animals treated with metronidazole and it has been proposed that as precautionary measure its use should be avoided during pregnancy.

Several natural products have amoebicidal activity, including the alkaloids emetine, cryptopleurine, berberine, matrine, cytosine and conessine. Hollarhena

antidysenterica (Kurchi bark) traditionally used for treatment of dysentery is commonly administered in the form of bark extract. Kurchi bismuth iodide consisting of the bismuth iodide of the mixed alkaloids of the bark has been used. Variable clinical results with Kurchi had been reported from French workers, which may be due to differing content of alkaloids in the fraction used in clinical studies. As a result, connesine, the principal alkaloid of Kurchi bark, was tried in amoebiasis and has been found to be effective, and many observers class connessine as equal in efficacy to emetine. Connessine has been found to be most effective against Entamoeba histolytica in both invitro and invivo screenings. Many of the drugs used currently are immunodepressant but connesine has been reported to have no effect on the immune system(Handa S.S ,1991).

Plant Bitters :

Among the various remedial agents from natural sources available to the medical science, bitters constitute as important group of substances. Quassinoids from Simaroubaceous plants, irridoid glycosides from Swertia and Picrorrhiza, alkaloid quinine from Cinchona and andrographolids from Andrographis paniculata constitute some of the most important plant bitters having wide spectrum of biological activity. Investigation of more plant bitters in future is likely to yield more bioactive phytoconstituents (Kinghorn A.D 1982).

Plant Adaptogens :

In Ayurvedic practice 'Rasayanas' are prescribed for longevity, rejuvenation and general well being. Two new acylsteryl glucosides-sitoindoside VII and sitoindoside VIII, isolated from roots of Withania somnifera showed antistress activity and were found to be uniformly effective in attenuating stress induced responses ranging from anxiety, depression, analgesia, thermal changes, gastric

ulcers, convulsions etc., and these findings tend to rationalize the use of the plant W. Somnifera as a ginseng substitute in Ayurveda. Future of such adaptogens, which can create immunity, is bright and we should search for adequate models to validate the claims of our indigenous drugs in this area (Mukerjee T.1984).

Plant Laxatives:

Constipation is a common problem of Western and European countries because of protein rich diet. In the U.S.A. 1000 patents of vegetable origin are there which involve and annual trade of $ 500 million. We export 15,000 tons of Plantago, 7000 tons of Senna and 1000 tons of Rhubarb annually. Trade in plant laxatives increases-by over 10% every year25.

Since disease decay and death have always co- exited with life, the study of diseases and their treatment must also have been con–temporaneous with the dawn the human intellect. The primitive man must have used as therapeutical agents and remedial measures those things which he was able procure most easily. There is no authentic record of medicines used by the primitive man. But the *Rig-Veda* that is the oldest book in the library of man supplies curious information on the subject. From it, we learn that the Indo-Aryans the *Soma* as a medicinal agent. It is not quite certain what the *soma* * plant was. This plant has not yet been satisfactorily identified. The Indo-Aryans used the plant for sacrificial purposes and its juice is described in the:

PLANT PROFILE

Carissa carandas Linn is a large evergreen shrub with a short stem, glabrous except the inflorescence; bark light gray, scaly; branch lets usually alternate, with thin stout sharp horizontal glabrous spines 2.5-3.8cm.long at their base; branches usually with out spines. Leaves 3.8-7.5 by 2.5-5.0cm, coriaceous, ellepatic or obovate, obtuse,

often shortly mucronate, glabrous and shining, base subacute; petioles 3.6mm long. Flowers white, scoarcely odorous, in pubescent terminal corymbose cymes; ped uncles usually 6.0-20mm long, sometimes almost zero. Pedicels very short; bracts linear, pubescent. Calyx pubescent, cleft rather more then ½ way down; lobes 2.5-3.0mm long, lanceolate, vary acute, celiate. Corolla tube about 1.3cmlong swollen and pubescent near the top; lobes 1cmlong, oblong-lanceolate, acute and pubescent. Stamens included within the corolla tube; filaments vary short; anthers linear-oblong. Ovary glabrous; ovules 4 in each cell; stigma slightly pencillate at the apex. Fruit 1.3-2.5cm long, ellipsoid, purplish black when ripe, moth 4 0r more seeded.

Synonym:

The shrub *Carissa carandas* Linn indifferent languages it is called as follows:

English : Karaunda, Jasmine flowered carissa

Hindi : Karaunta, Karoonti

Kannada : Krikayi

Malayalam : Klavu, Perumklavu, Karanta

Sanskrit : Karamaradh, Avighnah

Tamil : Kalakkai, Perumkla

Telugu : Vaka, Peddakalavi

Biological source:

It consists of dried root of the plant known as *Carissa carandas* Linn

Family:

It is belonging to family Apocynaceae

Geographical source:

Throughout India, in dry forests, also cultivated in Ceylon, Java, Timor.

Morphology of *Carissa carandas* root:

Color: reddish brown color

Odor: It has pleasant odor

Taste: It has characteristic and bitter taste

Size: About 12 to 15 cm long and 2 to 3 cm in diameter .

Shape: It is available in cylindrical form

Pharmacological Study:

The root of *Carissa carandas* was histological studied by Singh and Madan. Joglekar and Gaitonde reported its histamine releasing activity in 1970. The cardiotonic activity of C*arissa carandas* root methanolic extract was studied in 1963 by Vohra and De. Antipyretic activity was evaluated Rajasekaran and Jeyasudha.

Phytochemical Study:

Skarand and Francis tested the suitability as alternative sources renewable Energy and phytochemicals and its polyphenol, oil and hydrocarbon contents of *Carissa carndas* Linn were analyzed. One hundred and fifty constituents were identified; all of them were reported for the fist time as volatile components of karanda. A rough survey of the chemical classes represented in this fruit flavor was follows: alcohol comprised the largest class of volatile oil (29.8%), while the composition of the other classes of compound was as follows terpenoids 24.0 %; esters, 23.1%, fatty acids, 11.9 %; carbonyls, 2.2 %; falvonoids, 1.7 % and others 4.2 %. 4.

PHARMACOGNOSTIC STUDY

PHARMACOGNOSTIC STUDY

Carissa Carandas Linn is a large evergreen shrub with a short stem, glabrous except the inflorescence; bark light gray, scaly; branch lets usually alternate, with thin stout sharp horizontal glabrous spines 2.5-3.8 cm long at their base; branches usually with out spines. Leaves 3.8-7.5 by 2.5-5.0 cm, coriaceous, ellepatic or obovate, obtuse ,often shortly mucronate, glabrous and shining ,base subacute ;petioles 3.6mm long .Flowers white, scarcely odorous ,in pubescent terminal corymbose cymes ; ped uncles usually 6.0-20mm long, sometimes almost zero. Pedicels very short; bracts linear, pubescent. Calyx pubescent, cleft rather more then ½ way down; lobes 2.5-3.0mm long, lanceolate, vary acute, celiate. Corolla tube about 1.3cmlong swollen and pubescent near the top; lobes 1cmlong, oblong-lanceolate, acute and pubescent. Stamens included within the corolla tube; filaments vary short; anthers linear-oblong. Ovary glabrous; ovules 4 in each cell; stigma slightly pencillate at the apex. Fruit 1.3-2.5cm long, ellipsoid, purplish black when ripe, moth 4 0r more seeded (Fig 1).

Morphology of *Carissa carandas* root (Fig 2):

Color: reddish brown color

Odor: It has pleasant odor

Taste: It has characteristic and bitter taste

Size: About 12 to 15 cm long and 2 to 3 cm in diameter.

Shape: It is available in cylindrical form.

Materials and methods for anatomical studies

Collection of specimens:

The plant specimens for the proposed study were collected from in and around Bidar city .The specimen (plant) was identified by Prof. B.S.Sajjan, Hed, Dept.of Botany, B.V.B.Coolege, Bidar (Karnataka-INDIA). The care was taken to select healthy plants for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FFA (Farmalin-5ml+Acetic acid-%ml+70% ethyl alcohol-90ml). After 24hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the schedule given by saas; 1940.Infiltration of the specimens was carried by gradual addition of paraffin wax (Melting point 50-60°C) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

Sectioning:

The paraffin embedded specimens were section with the help of rotary Microtome. The thickness of the sections was 10-12µm. Dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with toluidine blue as per the method published by O'Brien et al.(1964) .Since Toluidine blue is a polychromatic stain .The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc.wherever necessary sections were also stained with safrannin and fast green and IKI (for Starch).

For studying the stomayol morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial

maceration employing Jeffrey's maceration fluid (Saas, 1940) were prepared. Glycerin mounted temporary preparations were made for macerated or clear materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell component were studied and measured.

Photomicrographs:

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab hot 2 microscopic Unit. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light were employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figure were indicated by scale-bar. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esau, 1964) (Fig 3-5).

Physico-chemical parameters:

Physico-chemical parameters were determined as per Ayurvedic Pharmacopoeia of India reported as total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive and moisture content.

Determination of ash value:

Ash value are used to determine quality and purity of crude drug .Ash contains inorganic radicals like phosphates, carbonates, and silicates of sodium, potassium, magnesium, calcium etc Some times variables like calcium oxalate, silica, carbonate content of the crude drug effect Total ash value; Such variables are then removed by treating with acid and then acid insoluble ash is determined heated with burner, using flame about 2 cm high and supporting the dish about 7 cm. above the flame, heat till

vapors almost cease to be evolved ; then lower the dish and heat more strongly until all the carbon is burnt off .cool in dessicator . Weighed the ash and calculated the percentage of total ash with reference to the air dried sample of the crude drug

Determination of total ash:

Sample was weighed and ignited in flat, thin, porcelain dish weigh about 2gm of powdered drug into the dish and cease to be evolved; then lower the dish and heat more strongly until all the carbon is burn off Cool in dessicator. Weighed the ash and calculated the percentage of total ash with reference to the air-dried sample of the crude drug.

Calculation

wt of the empty dish=X (19.01g)

wt of the drug taken=Y (2 g)

wt of dish +ash (after complete incineration)=Z(19.346g)

wt of ash = (Z-X)g(0.336g)

y g of the crude gives(Z-X)g of the ash=0.336g

100g of the drug gives 100/y X (Z-X)g of the ash

Total ash value of sample =100(Z-X)/y %

Total ash value of sample =100(0.336)/2% =16.8%

Total ash value of *Carissa carandas* root is 16.8%

Determination of acid insoluble ash:

Ash value are used to determine quality and purity of crude drug .Ash contains inorganic radicals like phosphates, carbonates, and silicates of sodium, potassium, magnesium, calcium etc Some times variables like calcium oxalate, silica, carbonate content of the crude drug effect Total ash value; Such variables are then removed by treating with acid and then acid insoluble ash is determined heated with burner ,using

flame about 2cm high and supporting the dish about 7cm.abovethe flame, heat till vapors almost cease to be evolved ; then lower the dish and heat more strongly until all the carbon is burnt off .cool in dessicator . Weighed the ash and calculated the percentage of total ash with reference to the air-dried sample of the crude drug.

Using 25 ml of dilute hydrochloric acid wash the ash from the dish used for total ash into a100ml beaker. placed wire gauge over a Bunsen burner and boiled for five minutes .filtered an ash less filter paper ,wash the residue twice with hot water .Ignited a crucible in the flame ,cool and weighed put the filter paper and residue together into the crucible ;heated gently until vapors cease to be evolved and then more strongly until all carbon has been removed .Cooled in dessicator .Weighed the residue and calculated acid –insoluble ash of the crude drug with reference to the air dried sample of the drug.

Calculation

Wt. of the empty dish (X) = 19.01gm

Wt. of the drug taken (Y) = 02.00gm

Wt. of dish +ash (after complete incineration) (Z) = 19.346

Wt. of ash (Z-X) = 19.346-19.01 = 0.336gm

2g of the crude gives (Z-X) g of the ash =0.336gm

100g of the drug gives 100/y X (Z-X)g of the ash

Total ash value of sample =100(Z-X)/y %

Wt of residue (obtained from total ash) (a) = 0.212gm

(Acid insoluble ash)

2gm of the air-dried drug gives 0.212gm acid insoluble ash 100gm of the air-dried drug gives 100 x a / y gm of acid insoluble ash. Acid insoluble ash value of the sample =100x0.212/2. Acid Insoluble ash value of the *Carissa Carandas* root is 10.6%

Determination of extractive values:

Extractive values are useful for the evaluation of a crude drug .gives idea about the nature of the chemical constituent present in a crude drug .Useful for the estimation of specific constituents ,soluble in particular solvent used for extraction

Determination of alcohol-soluble extractive:

Weighed about 5g of the powdered drug in a weighing bottle and transfer it to a dry 250 ml .conical flask .Filled a100 ml graduated flask to the delivery mark with solvent (90% alcohol).Wash out the weighing bottle and pour the washing ,together with the reminder of the solvent in to conical flask .Filtered in to a 50 ml cylinder .When sufficient filtrate has collected ,transfer 25 ml of the filtrate to a Weighed thin porcelain dish ,as used for the ash values determination .Evaporated to dryness on a water bath and complete the gives =4x sample = drying in an oven at 100^0C.Coolled in a desicccator and weighed .Calculated percentage w/w of extractive with reference to the air-dried drug

Calculation

25 ml of alcoholic extract gives =x g (0.089g) of residue

100 ml of alcoholic extract 4x g of residue =4 x 0.089 =0.356 g

5 g of air dried drug gives –4x g of alcohol (90%) soluble residue =0.356g

100 g of air dried drug gives –80x g of alcohol (90%) soluble residue =80 x 0.089=7.12g

Alcohol (90%) soluble extractive value of the sample =80x % = 7.12 %

Determination of water soluble extractives:

Weighed about 5g of the powdered drug in a weighing bottle and transfer it to a dry 250-ml. conical flask. Filled a100 ml graduated flask to the delivery mark with solvent (chloroform water). Wash out the weighing bottle and pour the washing, together with the reminder of the solvent in to conical flask. Filtered in to a 50 ml cylinder. When sufficient filtrate has collected, transfer 25 ml of the filtrate to a Weighed thin porcelain dish ,as used for the ash values determination .Evaporated to dryness on a water bath and complete the drying in an oven at 100°c. Cooled in a dessicator and weighed .Calculated percentage w/w of extractive with reference to the air-dried drug.

Calculation

25 ml of chloroform water extract gives =x g (0.08g) of residue

100 ml of chloroform water extract gives =4x0.08 g of residue=0.32g

5 g of air dried drug gives =4x g of chloroform water soluble residue

100 g of air dried drug gives =80x g of chloroform water soluble residue

chloroform water soluble extractive value of the 80x %=80x0.08=6.4%

Determination of moisture (loss on drying):

Weighed about 1.5 gm of the powdered drug into a weighed flat and thin porcelain dish. Dried in the oven at 100^0 C. Cooled in a dessicator and recorded weight of the sample .The loss in weight is usually recorded as moisture

Calculation

Wt of the empty dish (X) =56.77 g

Wt. the drug taken(Y) =1.5 gm

Wt of the empty dish(X) + wt. the drug taken(Y) =58.27 g

Wt of the dried sample + W.t of the empty dish = 58.6 g

the loss of weight = 58.27-58.26 g =0.1g

%of moisture content = 100/1.5 x 0.1 =6.66%

Anatomy of the root:

Both thick root and thin root were studied. Thin root **(Fig 3):**-Thin root measuring about 2.4mm was studied .t consists of crushed and obliterated ,dark surface layer followed by four or five layer of thin of thin walled,suberised phellum of periderm .A narrow secondary cortex phellum follows the priderm which consists of prenchyma cells the secondary xylem cylinder is solid and dense with wavy outline .It consists of diffusely distributed narrow as well as wide vessels and thick walled highly lignified. The narrow vessels are 50um in diameter and the wider vessels are 200um wide.

Thick root (fig 4and 5):

The root is 4.6mm in diameter .It has a wide periderm which is uniformly thickness around the root; it is deeply fissured; it consists of tangentially oblong thin walled suberised phellum cells. Secondary cortex is very narrow and the cells have been compressed due to tangential stress. Secondary phloem is wide and consists of outer zone of cursed and collapsed elements and inner narrow zone of intact non-collapsed elements. (fig 3.1). Secondary xylem cylinder is 3.3 mm thick it has many distinct growth rings with ring porous vessel distribution. The growth ring boundaries are de macerated by narrow with wider early wood vessels, arranged in longitudinal bonds (Fig 3.2), the vessels are variables in shape ranging circular, ovate to angular; the diameter of the metaxylam (wide vessels) is 150um,the narrow vessels are 40um .The xylem fibers are thin walled in the early wood portion thick walled in the late wood. The fibers are uniform type xylem rays are not distinct.

Root-macerate (fig 6and 7):

Macerated powder of the root exhibits various elements: - (1)Vessel elements(fig 6) :- Vessel elements are cylindrical ,elongated and tailed at both ends ,one end or tailless .They have simple ,circular and slightly inclined .The lateral wall pits are circular and dense .The length of the vessel elements is 400-650um (2)Traecheid. The traecheids are vessel like elements with dense lateral wall bordered pits but they ,do not have perforation plate .They are narrow and tapering at the ends .The tracheids are 300umlong. (3) Fiber-trachceids (Fig7);-These are narrow long fiber like cells with pointed ends and well developed lateral pits and thick walls .700um long . (4)Fibers (Fig 7);-The fibers are longer than the fiber tracheids; their walls are also thicker .They have single vertical row pits or pits may not be distinct .The fibers than 750um long.(5)Xylem parenchyma(Fig6):-Renangular ,wide ,thick walled parenchyma cells are the xylem parenchyma elements .They are storage in function with dense accumulation of starch grains

FIG 1:*CARISSA CARANDAS* LINN WHOLE PLANT

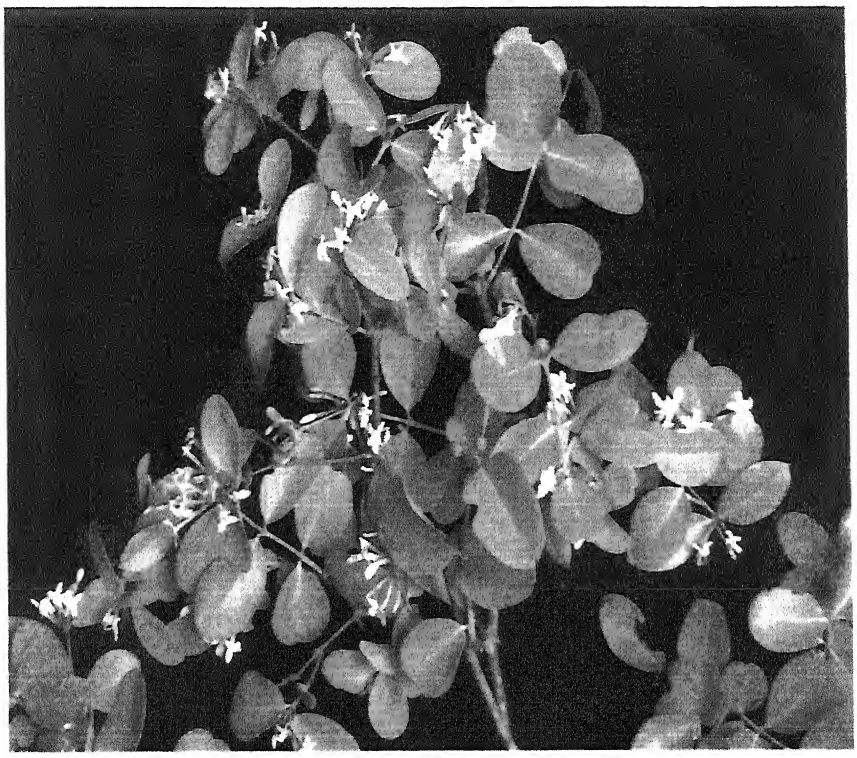


FIG.2: ROOTS OF CARISSA CARANDAS

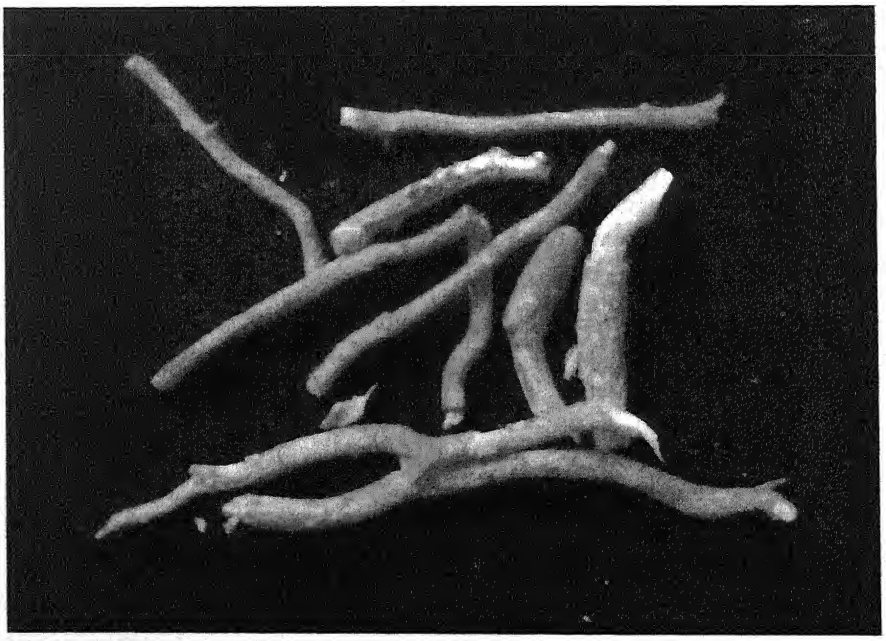
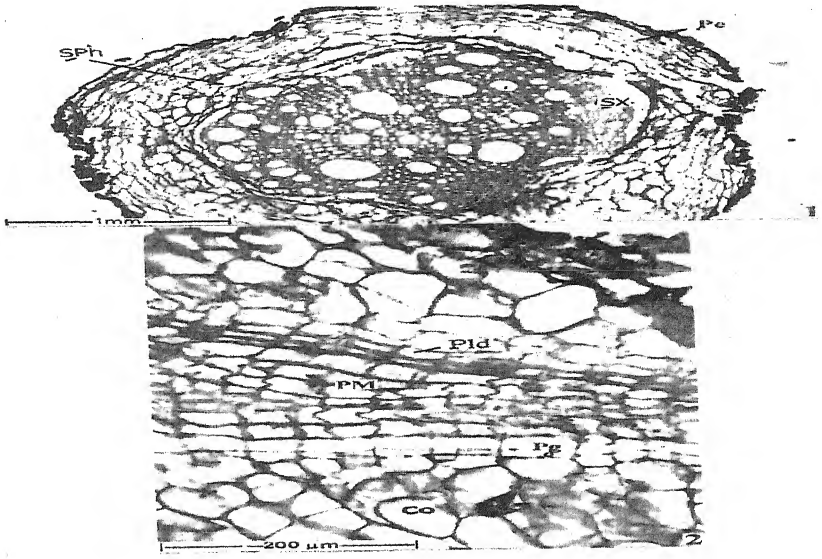
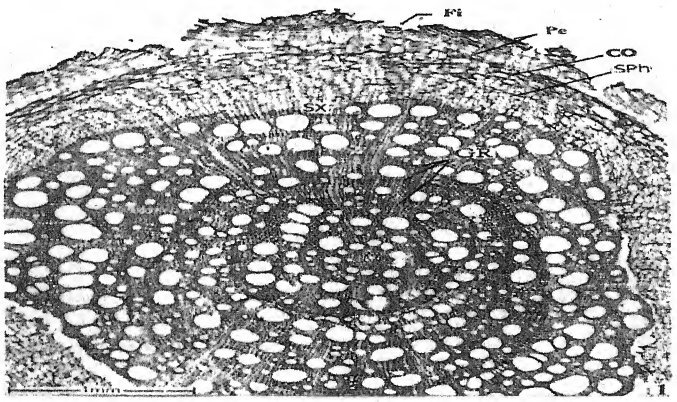


FIG: 3 ANATOMY OF THE THIN ROOT



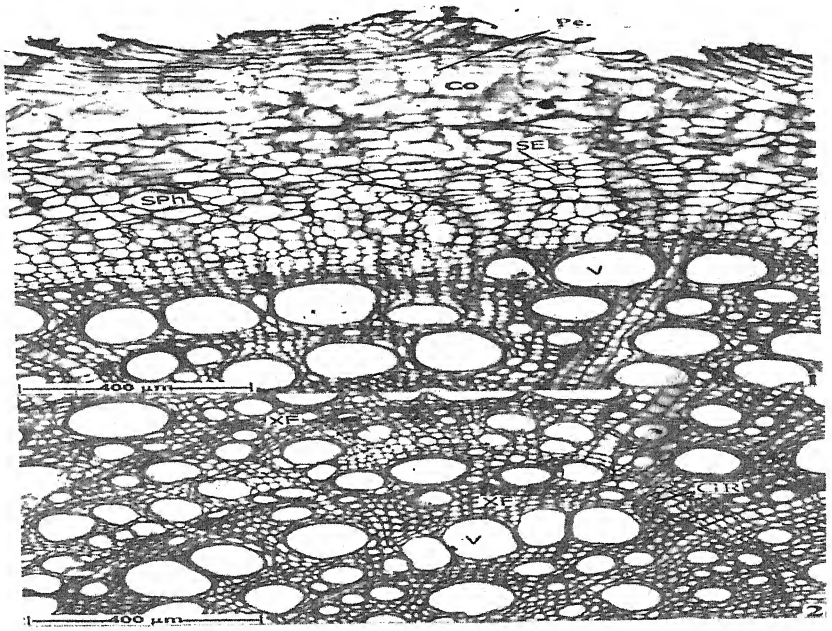
1. T.S of root ground –plane. 2. T.S of root a sector enlarged. (Co-Cortex; Pe =Peridem; pg=Phellogen; Pld=Phelloderm; Ph=Phellem; Sph=Secondary phloem; Sx=Secondary Xylem)

FIG 4. T.S OF THICK ROOT A SECTOR ENLARGED



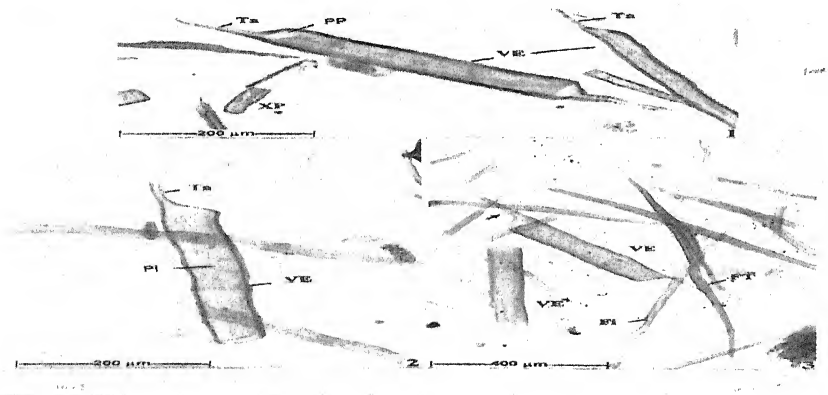
[Co=Cortex; Fi=Fissured; GR=Ground ring; PE =Periderm, Sph =Secondary phloem; Sx=secondary xylem]

FIG: 5: ANATOMY OF THE THICK ROOT



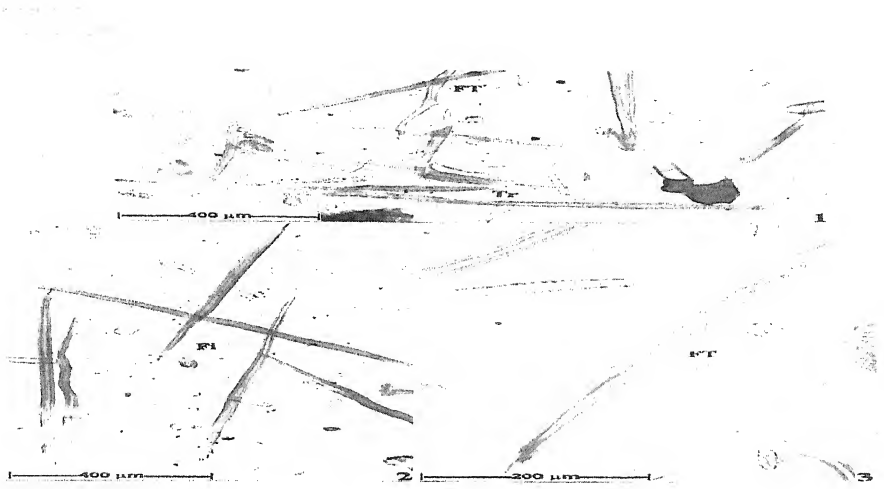
1: Secondary phloem and secondary xylem enlarged. 2: Structure of secondary xylem showing growth ring [Co= cortex; GR =Growth ring; Pe=Periderm; SE= Sieve element; Sph =Secondary phloem; V= Vessel; XF = Xylem Fibers]

FIG. 6: POWDER MICROSCOPY OF THE ROOT



1. Root Powdered tailed vessel element and xylem parenchyma cells. 2. Tailed vessel element magnified 3.Fibers, Fibre tracheids and Vessel element [Fi=fibre; FT= fibre tracheid; pi= Pits; PP= Perforation plate; Ta= Tail]

FIG .7: FIBERS AND FIBERS TRACHEIDS



1 fibers tracheid and tracheids. 2. Fibers. 3 fibers tracheids enlarged [Fi= Fiber; FT = fibers tracheids; Tr= trachieds]

RESULTS

Microscopical examination of *Carissa carandas* Root revealed the presence of phellum of the periderm, a narrow secondary cortex, secondary xylem showing growth ring, thick walled highly lignified narrow vessels, vessel elements, tracheids, fibre tracheids, fibre, xylem parenchyma. The T.S. of root showing Cortex; Peridem; Phellogen; Phelloderm; Phellem; Secondary phloem; Secondary Xylem; Fissured; Ground ring. The Secondary phloem and secondary xylem showing growth ring Periderm; Sieve element; Secondary phloem; Vessel; Xylem Fibers. The root Powdered showing tailed vessel element and xylem parenchyma cells, with fiber tracheids and Vessel elements (Fig. 3-7). The drug showed the presence of total ash 16.8%, acid insoluble ash 10.6%, water-soluble extractives 6.4%, alcohol soluble extractives 7.18% and moisture content 6.66%.

DISCUSSION

The morphological and microscopic studies of the present study has revealed the common characteristics of Apocynaceae as reported by earlier studies. The phsico-chemical analysis reported in the present study would be possibly used in future standardization and identification of the *Carissa carandas* root and its preparations. The results of this study could also be used in finding out reproducibility of the results of past studies and future studies and minimizing the error.

PHYTOCHEMICAL STUDY

PHYTOCHEMICAL STUDY

The plant was collected from forest in and around the Bidar and was authenticated by professor B.S.Sajjan Head Dept. of Botany, B.V.Bhoomraddi arts and sciences college, Bidar (Karnataka India). The roots of the plant *Carissa carandas* Linn was collected from forest in and around the Bidar in the month of June the collected material was immediately sprayed with ethanol to prevent the enzymatic degradation of secondary metabolites. The collected material was made into pieces by cutter mill (Inco-Make1995) to enhance the drying and to prevent the growth of fungus and microbes. The pieces of the root were dried under shade for 15-20 days.

The shade dried root material was powdered by using Hammer mill and hand grinder (Avishkar-suddal ,1995).About 5kg of the powder was charged in Soxhlet apparatus and was extracted with non polar solvents with increasing polarity (petroleum ether(40-60^0),benzene,chloroform, ethyl acetate and methanol) for 48hrs in fifty batches of 100gms for each batch. The extracts were collected to dryness in a rotary evaporator (Rolex-Mumbai) under reduced pressure and controlled temperature (50-60^{0}c). On concentration petroleum ether yielded light yellow gummy semisolid, benzene extract yielded yellow color gummy semisolid, chloroform extract yielded reddish brown color gummy semisolid, ethyl acetate extract yielded reddish brown color gummy semisolid, and, methanol extract yielded dark red color gummy semisolid and finally the residue air dried, reweighed and was macerated with chloroform water and extract was yielded reddish brown color gummy semisolid. After drying the above extracts weight of the each extract (100gms powder) was recorded and percentage yield was calculated (Table no 1)

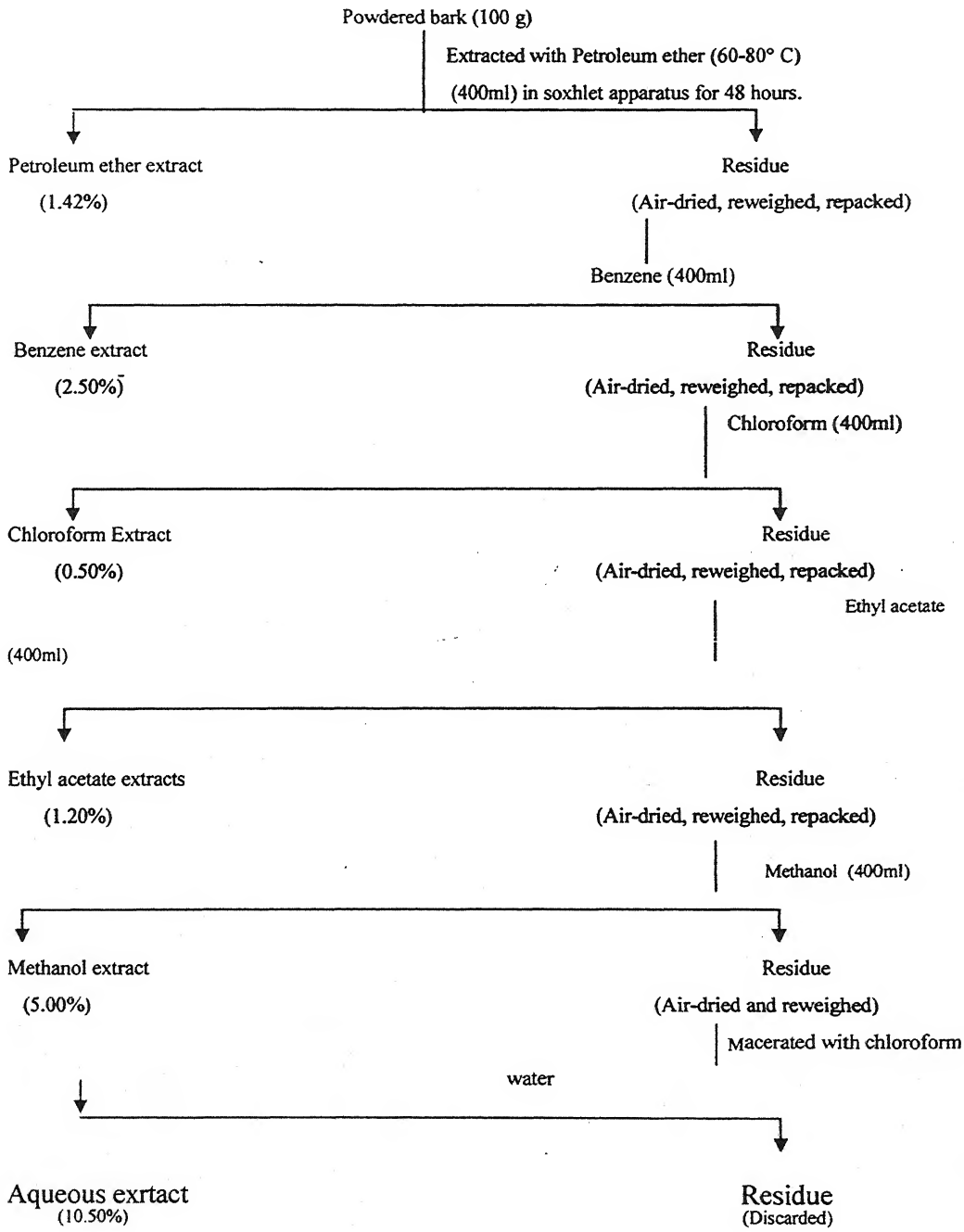
TABLE 1: NATURE AND PERCENTAGE YIELD OF VARIOUS EXTRACTS OF *CARISSA CARANDAS* ROOT

Root Extract	Nature	**Yield (in gms)**	**% Yield**
Petroleum ether Extract	Yellow gummy semisolid	1.42	1.42
Benzene extract	Yellow gummy semisolid	2.50	2.50
Chloroform extract	Reddish brown	0.50	0.50
Ethyl acetate extract	Reddish brown	1.20	1.20
methanol extract	Dark reddish	5.00	5.00
Aqueous extract	Reddish dark	10.5	10.5

The extracts were preserved in refrigerator and used for further investigation

SCHEME 1

Successive solvent extraction of Carissa carandas Root



PHYTOCHEMICAL STUDY OF EXTRACTS:

Phytochemical screening of the extracts of *Carissa carandas* root was carried out in order to know the class of organic compound presents in the different extracts of the root was selected for the study, which further facilities for the identification of active constituents and their isolation.

Preliminary phytochemical screening of various extracts of *Carissa carandas* root

The plant may be considered as biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, volatile oil, tannins, etc that exert a physiologic effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites systematic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents. Photochemical screening of the extracts of the root was carried out in order to know the class of organic compound presents in the different extracts of the root selected for the study, which further facilities for the identification of active constituents and their isolation.

Qualitative chemical examination of various extracts:

The extracts obtained above were subjected to qualitative chemical tests for the identification of various root constituents.[3]

Qualitative chemical examination of petroleum ether extract:

(1) Test for carbohydrates

a) Molisch,s test : -ve

b) Benedict,s test : -ve

c) Fehling test : -ve

(2) Test for alkaloids

a) Mayer,s test : +ve

b)Wagner,s test : +ve

c)Dragendroff,s test : +ve

d) Hager,s test : +ve

(3) Test for saponins :

a)With water shows foaming : +ve

b) With blood,rupture of RBC cells : +ve

c)With fish toxicity produced within five minutes : +ve

(4) Test for flavonoids

a) Shinod,s test : -ve

b) Zinc hydrochloric acid test -ve

c) With sodium hydroxide : -ve

(5) Test for steroids :

a) Liebermann Burchard,s test : +ve

b) Salkowski,s test : +ve

Qualitative chemical examination of benzene extract:

(3) Test for carbohydrates

a) Molisch,s test : +ve

b) Benedict,s test : +ve

c)Fehling test : +ve

(4) Test for alkaloids

a) Mayer,s test : +ve

b)Wagner,s test : +ve

c)Dragendroff,s test : +ve

d) Hager,s test : +ve

(3) Test for saponins :

a)With water shows foaming : +ve

b) With blood,rupture of RBC cells : +ve

c)With fish toxicity produced within five minutes : +ve

(4) **Test for flavonoids**

a) Shinod,s test : +ve

b) Zinc hydrochloric acid test +ve

c)With sodium hydroxide : +ve

(5) Test for steroids :

a) Liebermann Burchard,s test : -ve

b) Salkowski,s test : -ve

Qualitative chemical examination of chloroform extract:

(5) Test for carbohydrates

a) Molisch,s test : -ve

b) Benedict,s test : -ve

c)Fehling test : -ve

(6) Test for alkaloids

a) Mayer,s test : -ve

b) Wagner's test : -ve

c) Dragendroff,s test : -+ve

d) Hager's test : -ve

(3) Test for saponins :

a) With water shows foaming : -ve

b) With blood, rupture of RBC cells : -ve

c) With fish toxicity produced within five minutes : -ve

(4) **Test for flavonoids**

a) Shinod,s test : +ve

b) Zinc hydrochloric acid test +ve

c)With sodium hydroxide : +ve

(5) Test for steroids :

a) Liebermann Burchard,s test : -ve

b) Salkowski,s test : -ve

Qualitative chemical examination of ethyl acetate extract:

(7) Test for carbohydrates

a) Molisch,s test : +ve

b) Benedict,s test : +ve

c)Fehling test : +ve

(8) Test for alkaloids

a) Mayer,s test : -ve

b)Wagner,s test : -ve

c)Dragendroff,s test : -ve

d) Hager,s test : -ve

(3) Test for saponins :

a)With water shows foaming : +ve

b) With blood,rupture of RBC cells : +ve

c)With fish toxicity produced within five minutes : +ve

(4) **Test for flavonoids**

a) Shinod,s test : +ve

b) Zinc hydrochloric acid test +ve

c)With sodium hydroxide : +ve

(5) Test for steroids :

a) Liebermann Burchard,s test : -ve

b) Salkowski,s test : -ve

Qualitative chemical examination of methanol extract:

(9) Test for carbohydrates

a) Molisch,s test : +ve

b) Benedict,s test : +ve

c)Fehling test : +ve

(10) Test for alkaloids

a) Mayer's test : +ve

b) Wagner's test : +ve

c) Dragendroff,s test : +ve

d) Hager's test : +ve

(3) Test for saponins :

a) With water shows foaming : -ve

b) With blood,rupture of RBC cells : -ve

c) With fish toxicity produced within five minutes : -ve

(4) Test for flavonoids

a) Shinod,s test : +ve

b) Zinc hydrochloric acid test +ve

c) With sodium hydroxide : +ve

(5) Test for steroids:

a) Liebermann Burchard,s test : -ve

b) Salkowski, s test : -ve

Qualitative chemical examination of aqueous extract:

(11) Test for carbohydrates

a) Molisch,s test : +ve

b) Benedict,s test : +ve

c) Fehling test : +ve

(12) Test for alkaloids

a) Mayer's test : +ve

b) Wagner's test : +ve

c) Dragendroff,s test : +ve

d) Hager's test : +ve

(3) Test for saponins:

a) With water shows foaming: +ve

b) With blood, rupture of RBC cells : +ve

c)With fish toxicity produced within five minutes : +ve

(4) Test for flavonoids

a) Shinod,s test : +ve

b) Zinc hydrochloric acid test +ve

c)With sodium hydroxide : +ve

(5) Test for steroids :

a) Liebermann Burchard,s test : -ve

b) Salkowski,s test : -ve

Preparation of reagents for Phytochemical study :

Following reagents and solutions, which are required for the Photochemical Studies were prepared (Harborne, 1973).

Molisch's reagent:

10g of a-naphthol dissolved in 100 ml 95% ethanol

Authrone reagent:

0.2gm Of anthrone dissolved in 100 ml of concentrated H_2SO_4 and shaken (the solution should be freshly prepared).

Biuret reagent: 1.5 g of $CuSO_4$, 6g of sodium potassium tartarate were dissolved in 500ml of water, 300 ml freshly prepared NaOH solution was added to this solution and solution made upto the volume of one liter.

Ninhydrin reagent:

0.1% Ninhydrin dissolved in 100 ml of acetone.

Dragendroff,s reagent

50 g of tartaric acid was dissolved in 200ml of distilled water .4.5 g of basic bismuth nitrate was added and solution was shaken for two hours .100ml of 40% potassium iodide was then added and solution was shaken vigorously and allowed to stand for 24 hours and filtered

Mayer's reagent : 1.3 g mercuric chloride and 5g of potassium iodide were dissolved separately in 60mland 10ml of distilled water .

Phenolphthein reagent :

1g of Phenolphthein dissolved in 100ml ethanol

Lead acetate (10%)solution : 10 g of Lead acetate dissolved in 100ml methanol

Ferric chloride (5%)solution : 5g of Ferric chloride dissolved in 100ml ethanol

Alcoholic KOH(0.1N): 5.6 g of potassium hydroxide dissolved in 100ml ethanol

Gelatin Solution containing 10% NaCl : 1g of gelatin dissolved in 100ml of 10% Nacl

HCL solution : 50ml of con .HCL dissolved in 50ml of water

NaOH solution (10%): 10g NaOH dissolved in 100 ml of water

$CuSO_4$ solution : 24.7g of copper sulphate dissolved in 100 ml of water

Iodine in KI : Dissolve 20 g of KI and 12.7g of iodine in 30ml water diluted to one liter

Phytochemical testing and results Phytochemical tests for petroleum ether, benzene, chloroform, ethyl acetate and methanol extracts of *Carissa carandas* root extract were carried out as described by (Kokate 1985). The results of phytochemical tests are shown in **(Table no 3)**. Petroleum ether extract showed the positive tests for alkaloids, steroids, the benzene extracts showed the presence of alkaloids, saponins, and flavonoids, the chloroform extract showed the positive test proteins, saponins, ethyl acetate extract showed the positive tests for carbohydrates, saponins, and the methanol extract showed the positive tests for carbohydrates, proteins, alkaloids and flavonoids.

TABLE 2 : THE TYPE OF CHEMICAL CONSTITUENTS PRESENTING THE VARIOUS EXTRACTS OF *CARISSA CARANDAS* ROOT

Root Extract	Carbohyd Rates	Alkaloids	Saponins	Flavonoids	Steroids
Petroleum ether extract	-	+	+	-	+
Benzene extract	+	+	+	+	-
Chloroform extract	-	-	-	+	-
Ethyl acetate extract	+	-	+	+	-
methanol extract	+	+	-	+	-
Aqueous extract	+	+	-	+	+

Chromatographic processing of benzene extract

Benzene extract was subjected to thin layer chromatography aluminium sheet preparative TLC and using Solvent system benzene: ethyl acetate: chloroform (4:2:1) (Solvent system was selected by trial and error method).The plate was sprayed with dilute

sulphuric acid and observed under UV light showing five spots from top to towards bottom are named them band I, band II ,band III, band IV, and band V.(Gurrdeep,R Chatwal,1979) (Fig no 8)

Column chromatography of benzene extract

The benzene was chromatographed in a column with a aluminium oxide neutral built in petroleum ether and eluted with benzene :ethyl acetate :chloroform (4:2:1). Five fractions were collected and concentrated. Fraction –1 upon concentration yielded reddish semisolid mass (0.3g) and named them as B_1.Similarly fractions 2,3,4,and 5 are concentrated and yielded (0.2 g ,0.25g ,0.3g and o,2g respectively) [5] .They were labeled as B2, B3, B4, and B5 respectively . The collected fractions are subjected to thin layer chromatography using the solvent system benzene: ethyl acetate: chloroform (3:2:1), spraying the developed plates with dilute sulphuric acid yellow color spots were observed. The were calculated B_1 R_f value =0.45, B_2 R_f value = 0.58, B_3 R_f value= 0.93, B_4 R_f value = 0.62 and B5 R_f value =0.559 (Fig no 9) (Roger E,Schirmer)

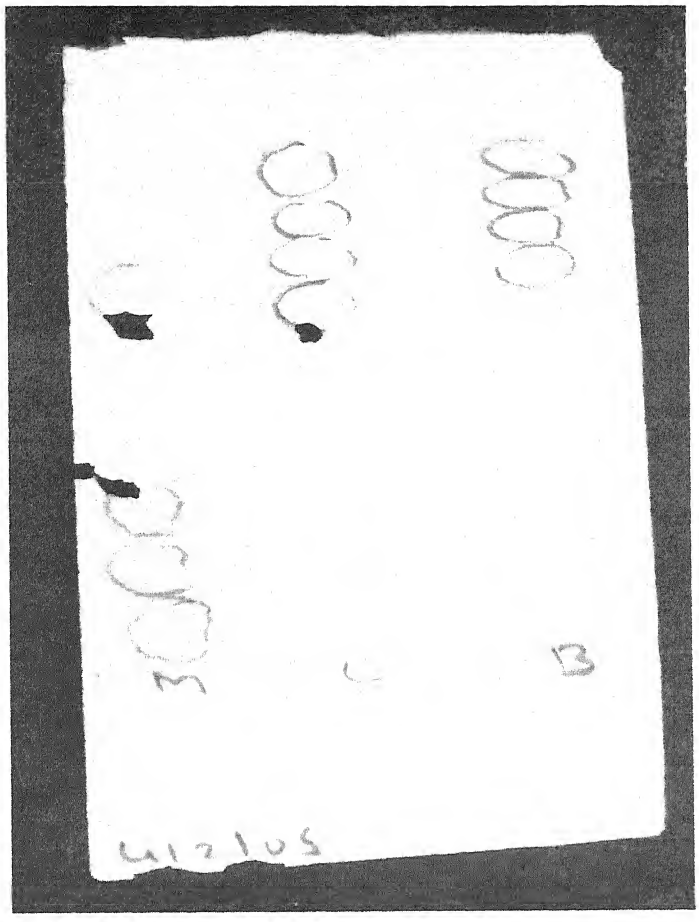


FIG. NO. 8 : TLC OF METHANOL, CHLOROFORM AND BENZENE EXTRACTS.

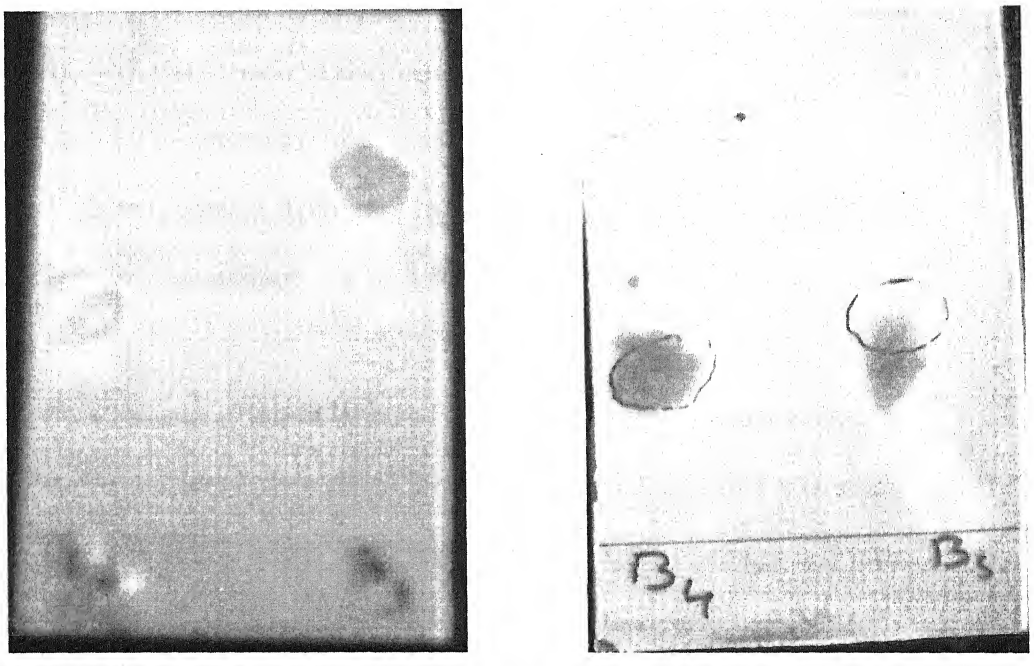


FIG. NO. 9: TLC OF BENZENE FRACTIONS B_2 B_3 B_4 & B_5

Detection of elements:

The first step in analysis of an organic compound is the detection of elements present in it. Most of the compounds contain 2 to 5 elements. The principal elements present are: carbon, hydrogen and oxygen. Often, in addition to these, they may contain nitrogen, sulfur and halogens. Phosphorus and metals are also present but only rarely. The order of abundance in which these elements are found in organic compounds is indicated below:

- Carbon → Always present
- Hydrogen → Nearly always present
- Oxygen → Generally present
- Nitrogen, Halogen and sulphur → Less commonly present
- Phosphorus and metals → Rarely present

Detection of carbon and hydrogen

If the compound under investigation is known to be organic, there is no need to test for carbon. This test is performed only to establish whether a given compound is organic or not. With the exception of few compounds eg. Carbon tetrachloride, all organic compounds also contain hydrogen. The presence of both these elements is controlled by the following common test. (B.S Bahal ,Arun Bahal)

Copper oxide test.

The organic substance is mixed intimately with about three times its weight by dry cooper oxide. The mixture is then placed in a hard glass test tube fitted with a bent delivery tube, the other end of which is dipping into limewater in another test tube. The mixture is heated strongly when the following reaction takes place:

$$C + 2CuO \rightarrow CO_2 + 2Cu$$

$$2H + CuO \rightarrow H_2O + Cu$$

Thus if carbon is present, it is oxidized to carbon dioxide which turns lime water milky. If hydrogen is also present, it will be oxidized to water which condenses in small droplets on the cooler wall of the test tube and inside the bulb the formation of water is further confirmed by testing the condensed liquid with anhydrous copper sulphate (white) that is turned blue.

Detection of oxygen:

There is no conclusive test for oxygen, through its presence in organic compounds is often inferred by indirect methods.

(1) The substance is heated alone in a dry test tube, preferably in an atmosphere of nitrogen. Formation of droplet of water on cooler part of the tube obviously shows the presence of oxygen. A negative result however, does not, necessarily show the absence of oxygen.

(2) The second method is to test for the presence of various oxygen containing groups such as OH, COOH,-NO_2 etc. If any of these is detected, the presence of oxygen is confirmed.

(3) The sure test for oxygen on the determination of the percentage of all other elements present in a given compound. If the sum of these percentages falls short of hundred, the remainder gives the percentage of oxygen and thus confirms its presence.

Detection of nitrogen

The presence of nitrogen in an organic compound is shown by the following test:

(1) A little of the substance is heated strongly in a test tube or by directly placing in the Bunsen flame. A smell of burnt feathers indicates nitrogen

(2) Soda lime test.

The given substance is mixed with the double the amount of soda lime and heated in a test tube. The vapors of ammonia evolved show the presence of nitrogen. A negative result, however not a proof of the absence of nitrogen. Many classes of nitrogen compounds including nitro and diazo derivatives do not respond to this test.

(3) Sodium test.

This is a good test for the detection of nitrogen in all classes of nitrogenous compounds. It involves the following steps;

(i) The substance is heated strongly with sodium metal.

$$Na + \underset{\text{(From org.Compd.)}}{C + N} \rightarrow NaCN$$

(ii) The water extract of the fused mass is boiled with ferrous sulphate solution.

$$FeSO_4 + 2NaOH \rightarrow Fe(OH)_2 + Na_2SO_4$$

$$6NaCN + Fe(OH)_2 \rightarrow Na4(Fe(CN)_6) + 2NaOH$$

(iii) To the cooled solution is then added a little ferric chloride solution and excess of concentrated hydrochloric acid.

$$Na4(Fe(CN)_6) + 4FeCl_3 \rightarrow FeCl_3(Fe(CN)_6)_3 + 12NaCl$$

The formation of Prussian blue or green coloration confirms the Presence of nitrogen.

Detection of sulphur

The presence of sulphur in an organic compound is shown as described below.

(i) Sodium test.

The given organic compound, upon fusion with sodium reacts to form sodium sulphide.

$$2Na + S \rightarrow Na_2S$$

Thus, the sodium extract obtained from the fused mass obtained may be tested as; (i) to a portion, add freshly prepared nitropruside solution. A deep violet color indicates sulphur

(ii) Acidify a second portion of the extract with acetic acid and then add lead acetate solution. A black precipitate of lead sulphide confirms the presence of sulphur.

$$Pb(CH_3COO)_2 + Na_2S \rightarrow PbS + 2CH_3COONa$$

(2) Oxidation test

The organic substance is fused with a mixture of potassium nitrate and sodium carbonate. The sulfur if present is oxidized to sulphate.

$$Na_2CO_3 + S + 3O \rightarrow Na_2SO_4 + CO_2$$

The fused mass is extracted with water, acidified with hydrochloric acid and then barium chloride solution is added to it. A white precipitate indicates the presence of sulfur.

$$BaCl_2 + Na_2SO_4 \rightarrow BaSO_2 + NaCl$$

Detection of halogens

(i) Sodium test.

Upon fusion with sodium, halogen in organic compound is converted to the corresponding sodium halide. Thus,

$$Cl + Na \rightarrow NaCl$$

$$Br + Na \rightarrow NaBr$$

$$I + Na \rightarrow NaI$$

Acidify a portion of extract with dilute nitric acid and add to it silver nitrate solution. White precipitate soluble in ammonia indicates chloride. Yellow precipitate sparingly soluble in ammonia indicates bromine. Yellow precipitate insoluble in ammonia indicates iodine.

(ii) Copper wire test.

The copper wire flattened at one end is heated in an oxidizing Bunsen flame till it ceases to impart any green color to the flame. A small quantity of the substance under investigation is now taken on the flattened end of the wire, which is re-inserted in the Bunsen flame. Upon heating for a while, halogen present in the substance is converted to a volatile copper halide, which imparts a blue or green color to the flame. This test though very sensitive, is not always reliable. A substance like urea which contains no halogen, also colors the flame green.

Detection of phosphorus

The solid substance is heated strongly with an oxidizing agent such as conc. nitric acid or a mixture of sodium carbonate and potassium nitrate. The phosphorus present in the substance is thus oxidized to phosphate. The residue is extracted with water, boiled with some nitric acid, and then a hot solution of ammonium molybdate is added to it in excess. A yellow coloration or precipitate indicates the presence of phosphorus.

The Benzene extract fractions such as B_1containing carbon, hydrogen, and oxygen, B_2 containing carbon ,hydrogen, and oxygen, B_3 containing carbon, Hydrogen, and oxygen, B_4 containing carbon ,hydrogen, oxygen and nitrogen and B_5 fraction containing carbon , hydrogen , oxygen and nitrogen .

Methanol extract fractions such as M_1 containing carbon, hydrogen, oxygen, and nitrogen, M_2 fraction containing carbon, hydrogen, oxygen and nitrogen, M_3 fraction containing carbon, hydrogen, oxygen and nitrogen M_4 fraction containing carbon, hydrogen and oxygen (Table no 2)

TABLE 3: TYPE OF ELEMENTS PRESENT IN FRACTIONS OF BENZENE EXTRACT AND METHANOL EXTRACT

	ELEMENTS				
Sample code	**Carbon**	**Hydrogen**	**Oxygen**	**Nitrogen**	**Phosphorus**
B1	+	+	+	-	-
B2	+	+	+	-	-
B3	+	+	+	-	-
B4	+	+	+	+	-
B5	+	+	+	+	-
M1	+	+	+	+	-
M2	+	+	+	+	-
M3	+	+	+	+	-
M4	+	+	+	-	-

Where: + indicates presence of element in a compound

: – indicates absence of element in a compound

Spectral studies of benzene extract: All the above five fractions were subjected to spectral analysis of IR, LC- MS, ^{1}HNMR.

Spectral analysis of component–B_2: (Fig. No.11) IR (in KBr) cm $^{-1}$

3466 (Hydrogen bonding, OH) 2852 - 2923 (C-H Stretching of CH_2 group) 1746 (C=O group) [6].

(2) ^{1}HNMR : (Fig No.12) ^{1}HNMR Spectrum of component B_2 reveals the absence of aromatic protons as there is no multiplate between delta 6.50-8.00 the peak at 7.3 which is singlet may be due to OH group . The peaks at delta 0.60-2.80 and 4.0-5.40 may be due to CH_2 and CH_3 group. From the study of IR and ^{1}HNMR spectra the component appears to be a fatty acids .In the IR spectrum the of compound the peak at 3466 may due to OH of COOH group the absence of characteristic band between 3000-3100 indicates the absence of aromatic C-H stretching and hence absence of aromatic ring . The peak at 2852-2923 represents C-H stretching of CH_2 and CH_3 groups. The peak at 1746is due to C=O group. These points suggests that the carboxyl group, metylene and methyl groups indicating that compound B_2 may be along chain fatty acid. Further ^{1}HNMR spectrum of the also supports fact that the compound B_2 may a fatty acid. The OH peak of COOH group splitting at delta at 7.3 and the CH_2 and CH_3 group splitting as triplet, quartet in the range between delta 0.60 – 5.40 hence the compound B_2 may be fattyacid.[7]

(3) LC-MS of B_2 compound: (Fig. No. 13) The LC-MS(Fig no) the mass spectrum of compound B_2 shows that the base peak is at m/z 413.1 and but the compound B_2 molecular peak has not recorded .The base peak indicates that the molecule contain high molecular weight compound as per the IR and ^{1}HNMR results the compound may be saturated fatty acid [8]

Spectral analysis B_3 compound : (Fig. No. 14) (1) IR (in KBr) cm^{-1}

3466 (Hydrogen bonding, OH) 2858 (C- H stretching of CH_2 group) 1708(C=O) group 1609 (C=C)

^{1}HNMR : (Fig. No. 15) ^{1}HNMR Spectrum of component B_3 reveals the absence of aromatic protons as there is no multiplate between delta 6.50 – 8.00 the peak at 7.3 which is singlet may be due to OH group . The peaks at delta 0. 60-2.80 and 4.0-5.40 may be due to CH_2 and CH_3 group. From the study of IR and ^{1}HNMR spectra the component appears to be a fatty acids. In the IR spectrum the of compound the peak at 3466 may due to OH of COOH group the absence of characteristic band between 3000-3100 indicates the absence of aromatic C-H stretching and hence absence of aromatic ring. The peak at 2852-2923 represents C-H stretching of CH_2 and CH_3 groups. The peak at 1708 is due to C=O group. These points suggests that the carboxyl group, methylene and methyl groups indicating that compound B_3 may be along chain fatty acid. Further ^{1}HNMR spectrum of the also supports fact that the compound B_3 may a fatty acid. The OH peak of COOH group splitting at delta at 7.3 and the CH_2 and CH_3 group splitting as triplet, quartet in the range between delta 0.60–5.40 hence, the compound B_3 may be unsaturated fatty acid. From the study of IR and ^{1}HNMR spectra of component B_3 appears to unsaturated long chain fatty acids as the characteristic absorbance band at 1609 in the IR spectrum of B_3 indicates the presence of unsaturation (C=C). This was further confirmed by chemical test as the compound B_3 showed decolorization with $KMnO_4$ and bromine water indicating the presence of unsaturation. Hence, the possible compound may be long chain unsaturated fatty acids.

LC-MS of B_3 compound: (Fig. No. 16)

The mass spectrum of compound B3 shows that the base peak is at m/z 242.3 and but the compound B_2 molecular peak has not recorded .The base peak indicates that the molecule contain high molecular weight compound as per the IR and ^{1}HNMR results the compound may be unsaturated fatty acid.

Spectral analysis of B_5: (Fig. No. 17) The IR spectrum of component B_5 showed the characteristic absorbance in the following regions. The broad peak at 3442 cm^{-1}may be due to hydrogen bonded NH2 group, the weak band around 3000-3100 cm^{-1} is due to aromatic stretching. The peak in the range 2400-2923 cm^{-1} may be due to C-H stretching of the CH_2 and CH_3 groups. The peak at 1732 cm^{-1} is due to C=O group. The peak at 1652 cm^{-1} may be due to N-H bending and C=N the peak at 1600 $cm^{-1}$is due to C= C.

^{1}HNMR spectrum of component B_5: (Fig. No. 18) ^{1}HNMR of the component B_5 showed the characteristics signals as below delta 0.6–3.0 and 3.5–4.5 indicates the presence of CH_2 and CH_3 groups. The peak at 5.1 may be due to NH_2 group and the peak at 6.7–7.7 may be due to aromatic proton indicating the presence of aromatic ring. The IR and ^{1}HNMR spectral studies of component B_5 suggests the presence of aromatic ring, amino group, carbonyl group, and methylene and methyl group. Hence, the compound B_5 may contain an aromatic with aside chain having CH_2 and CH_3 groups and containing amide group. Hence the compound B_5 is an aromatic amide.

LC-MS of B_5 compound: (Fig. No 19)

The mass spectrum of compound B_5 shows that the base peak is at m/z 803.6 but the compound B_5 molecular peak has not recorded .The base peak indicates that the molecule as per the IR and ^{1}HNMR results the compound may be aromatic amide.

Chromatographic processing of methanol extract: Methanol extract was subjected to thin layer chromatography aluminum sheet preparative TLC and using solvent system

benzene: ethyl acetate: chloroform (4:2:1) (Solvent system was selected by trial and error method). The plate was sprayed with dilute sulphuric acid and observed under UV light showing four spots from top to towards bottom are named them band I, band II, band III and band IV. (Fig no 8)

Column chromatography of methanol extract: The methanol was chromatographer in a column with a aluminum oxide neutral built in petroleum ether and eluted with benzene: ethyl acetate: chloroform (4:2:1). Four fractions were collected and concentrated. Fraction-1 upon concentration yielded reddish semisolid mass (0.2g) and named them as M_1. Similarly, fractions 2, 3 and 4 were concentrated and yielded (0.25 g, 0.3 g and 0.35 g respectively). They were labeled as M_2, M_3 and M_4 respectively. The collected fractions were subjected to thin layer chromatography using the solvent system benzene: ethyl acetate: chloroform (3:2:1), spraying the developed plates with dilute sulphuric acid, yellow color spots were observed. The R_f were calculated M_1 R_f value =0.85, M_2 R_f value =0.73, M_3 R_f value= 0.68, M_4 R_f value =0.65(Fig no10).All the four fractions were subjected to spectral analysis IR, ^{1}HNMR.and LC- MS

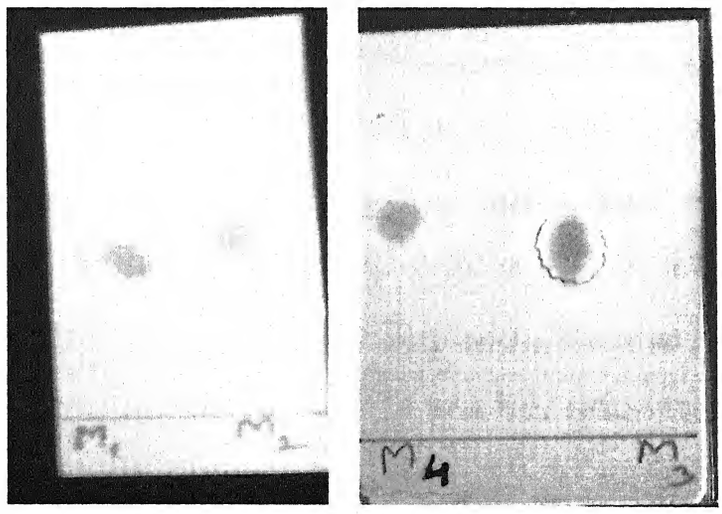


FIG. NO. 10: TLC OF METHANOLIC EXTRACT FRACTIONS M_1, M_2, M_3, & M_4.

Spectral studies of methanolic extract:

Spectrum of M_1 component: (Fig. No. 20) The IR spectrum of component M_1 showed the characteristic absorbance in the following regions. The broad peak at 3460 cm^{-1} may be due to hydrogen bonded NH_2 group; the weak band around 3000-3100 cm^{-1} is due to aromatic stretching. The peak in the range 2300-2919 cm^{-1} may be due to C-H stretching of the CH_2 and CH_3 groups. The peak at 1706 cm^{-1} is due to C=O group. The peak at 1652 cm^{-1} may be due to N-H bending and C=N the peak at 1608 cm^{-1} is due to C= C.

^{1}HNMR spectrum of component M_1: (Fig. No. 21) ^{1}HNMR of the component M_1 showed the characteristics signal as below delta 0.6-4.3 indicates the presence of CH_2 and CH_3 groups. The peak at 5.5 ppm may be due to NH_2 group and the peak at 7.2-7.7 ppm may be due to aromatic proton indicating the presence of aromatic ring. The IR and ^{1}HNMR spectra studies of component M_1 suggests the presence of aromatic ring, amino group, carbonyl group and methylene and methyl group. Hence, the compound M_1 may contain an aromatic with a side chain having CH_2 and CH_3 groups and containing amide group. Therefore, the compound M_1 is an aromatic amide.

. LC-MS of M_1 compound (Fig. No. 22)

The mass spectrum of compound M_1 shows that the base peak is at m/z 460.0 but the compound M_1 molecular peak has not recorded .The base peak indicates that the molecule as per the IR and ^{1}HNMR results the compound may be aromatic amide

Spectrum of M_2 component: (Fig. No. 23) The IR spectrum of component M_2 showed the characteristic absorbance in the following regions. The broad peak at 3460 cm^{-1} may be due to hydrogen bonded NH_2 group the weak band around 3000-3100 cm^{-1} is due to aromatic stretching. The peak in the range 2300-2919 cm^{-1} may be due to C-H stretching

of the CH_2 and CH_3 groups. The peak at 1706 cm^{-1} is due to C=O group. The peak at 1652 cm^{-1} may be due to N-H bending and C=N the peak at 1608 cm^{-1} is due to C= C.

^{1}HNMR spectrum of component M_2: (Fig. N0. 24) ^{1}HNMR of the component M_1 showed the characteristics signals as below delta 0.6-4.3 indicates the presence of CH_2 and CH_3 groups. The peak at 5.5 ppm may be due to NH_2 group and the peak at 7.2–7.7 ppm may be due to aromatic proton indicating the presence of aromatic ring. The IR and ^{1}HNMR spectral studies of component M_2 suggests the presence of aromatic ring, amino group, carbonyl group and methylene and methyl group. Hence, the compound M_2 may contain an aromatic with a side chain having CH_2 and CH_3 groups and containing amide group. Therefore, the compound M_2 is an aromatic amide.

. LC-MS of M_2 compound: (Fig. N0. 25)

The mass spectrum of compound M_2 shows that the base peak is at m/z 460.0 but the compound M_2 molecular peak has not recorded .The base peak indicates that the molecule as per the IR and ^{1}HNMR results the compound may be aromatic amide

Spectrum of M_3 component: (Fig. N0. 26) The IR spectrum of component M_3 showed the characteristic absorbance in the following regions. The broad peak at 3460 cm^{-1} may be due to hydrogen bonded NH_2 group the weak band around 3000-3100 cm^{-1} is due to aromatic stretching. The peak in the range 2300-2919 cm^{-1} may be due to C-H stretching of the CH_2 and CH_3 groups. The peak at 1706 cm^{-1} is due to C=O group. The peak at 1652 cm^{-1} may be due to N-H bending and C=N the peak at 1608 cm^{-1} is due to C= C.

^{1}HNMR spectrum of component M_3: (Fig. N0. 27) ^{1}HNMR of the component M_1 showed the characteristics signals as below delta 0.6– 4.3 indicate the presence of CH_2 and CH_3 groups. The peak at 5.5 ppm may be due to NH_2 group and the peak at 7.2–7.7 ppm may be due to aromatic proton indicating the presence of aromatic ring. The IR and The IR and ^{1}HNMR spectra studies of component M_3 suggests the presence of aromatic

ring, amino group, carbonyl group and methylene and methyl group. Hence, the compound M_2 may contain an aromatic with a side chain having CH_2 and CH_3 groups and containing amide group. Therefore, the compound M_3 is an aromatic amide.

LC-MS of M_3 compound (Fig. N0. 28)

The mass spectrum of compound M_3 shows that the base peak is at m/z 413.5 but the compound M_3 molecular peak has not recorded .as per the IR and ^{1}HNMR results the compound may be aromatic amide

NABL

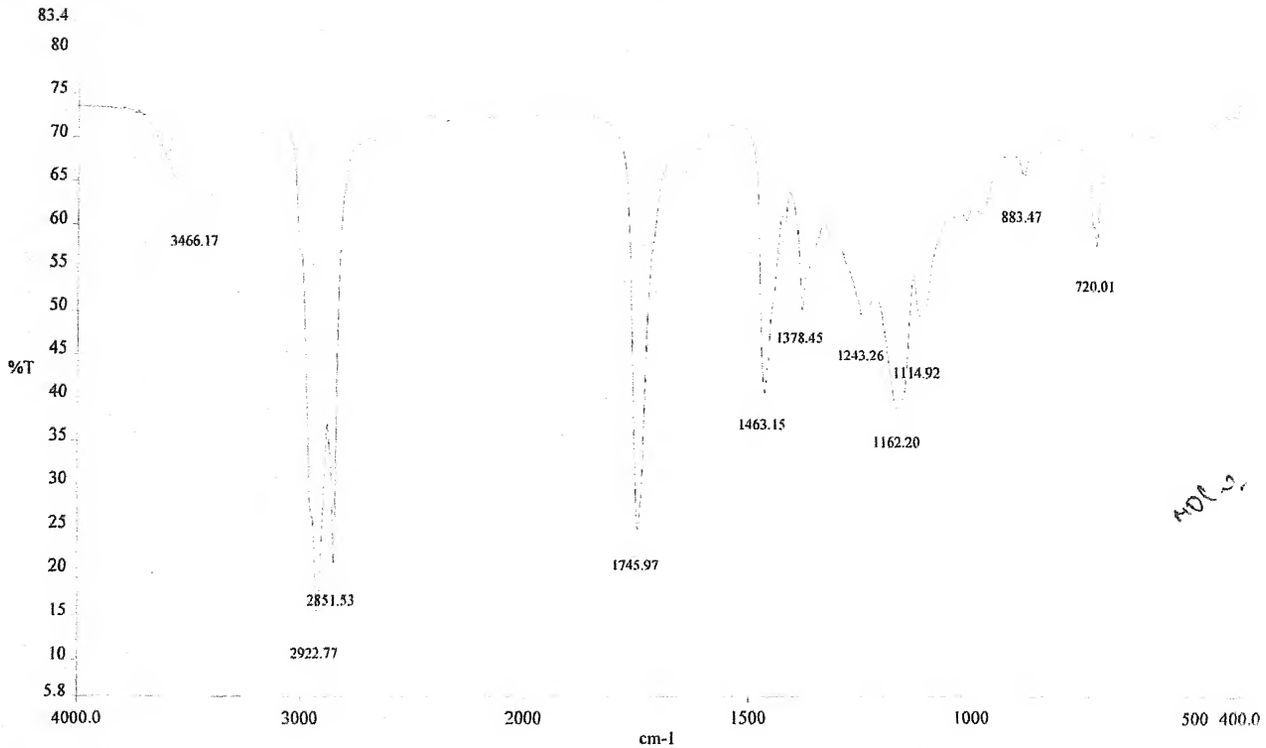
FTIR Spectrum

SIPRA LABS

Instrument Serial Number: 72425

Resolution: 4.00 cm-1

Date: 09-8-06



Spectrum Name: NP-1459-2.002

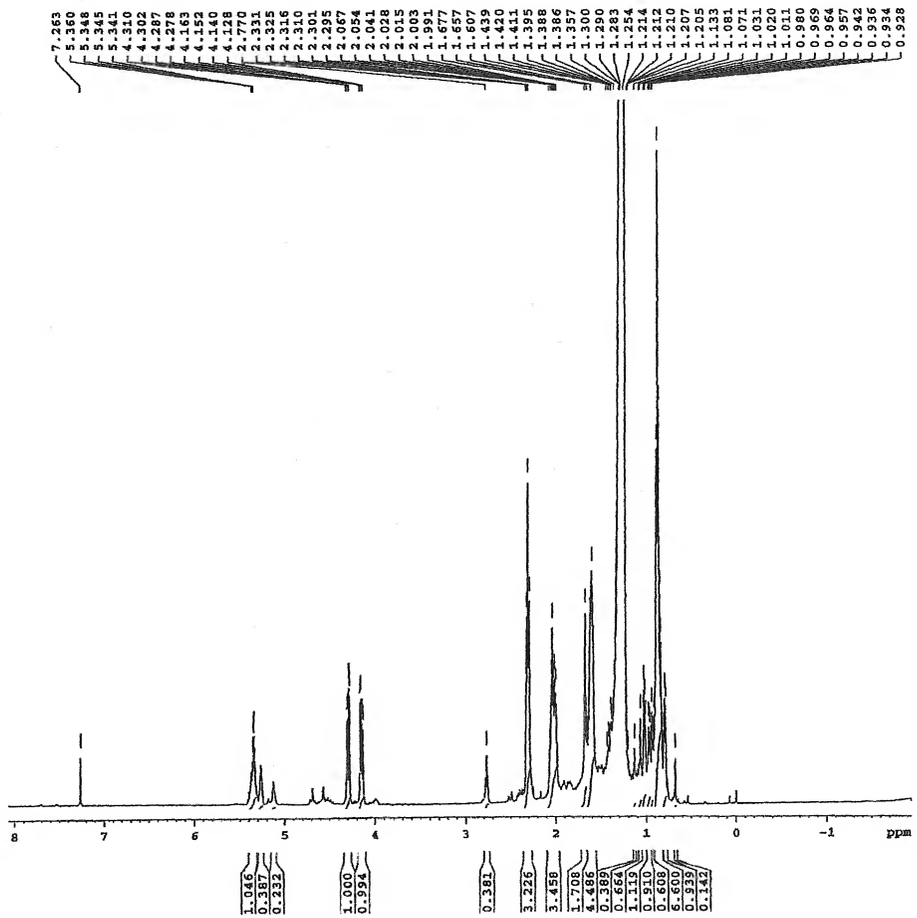
Description: EXTRACT B2,B2

Comments: KARNATAKA COLLEGE OF PHARMACY

Spectrum Pathname: C:\pel_data\spectra\NP-1459-2.002

70

Fig. No. 11



B 2

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PROCNO 1

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PULPROG zg
TD 16384
SOLVENT CDCl3
NS 256
DS 0
SWH 9765.625 Hz
FIDRES 0.596046 Hz
AQ 0.8389108 sec
RG 20.2
DW 51.200 usec
DE 6.00 usec
TE 297.9 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.52 usec
PL1 0.00 dB
SFO1 500.0339219 MHz

F2 - Processing parameters
SI 65536
SF 500.0300093 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

Fig. No. 12

,t of window 80: MS Spectrum

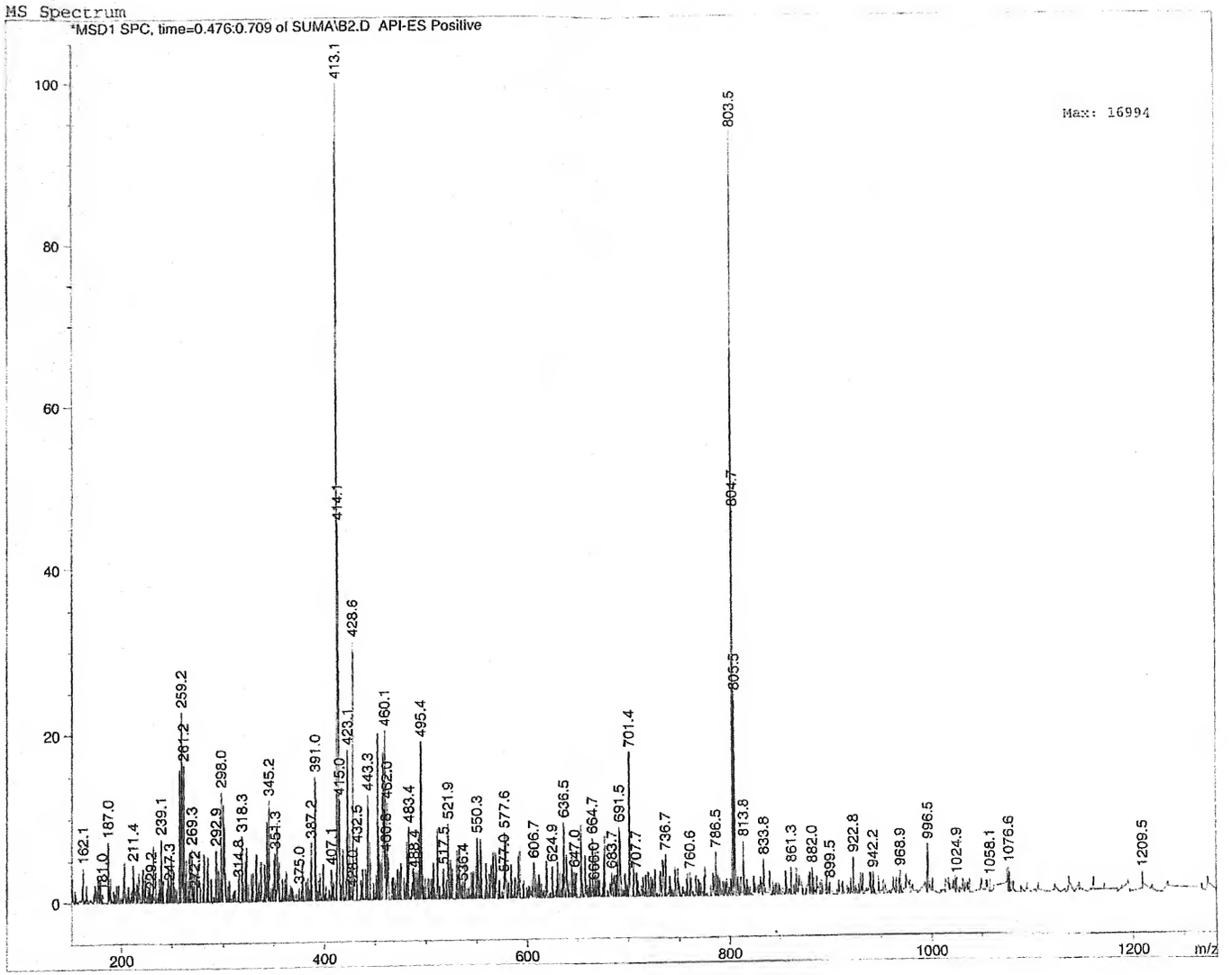


Fig. No. 13

NABL

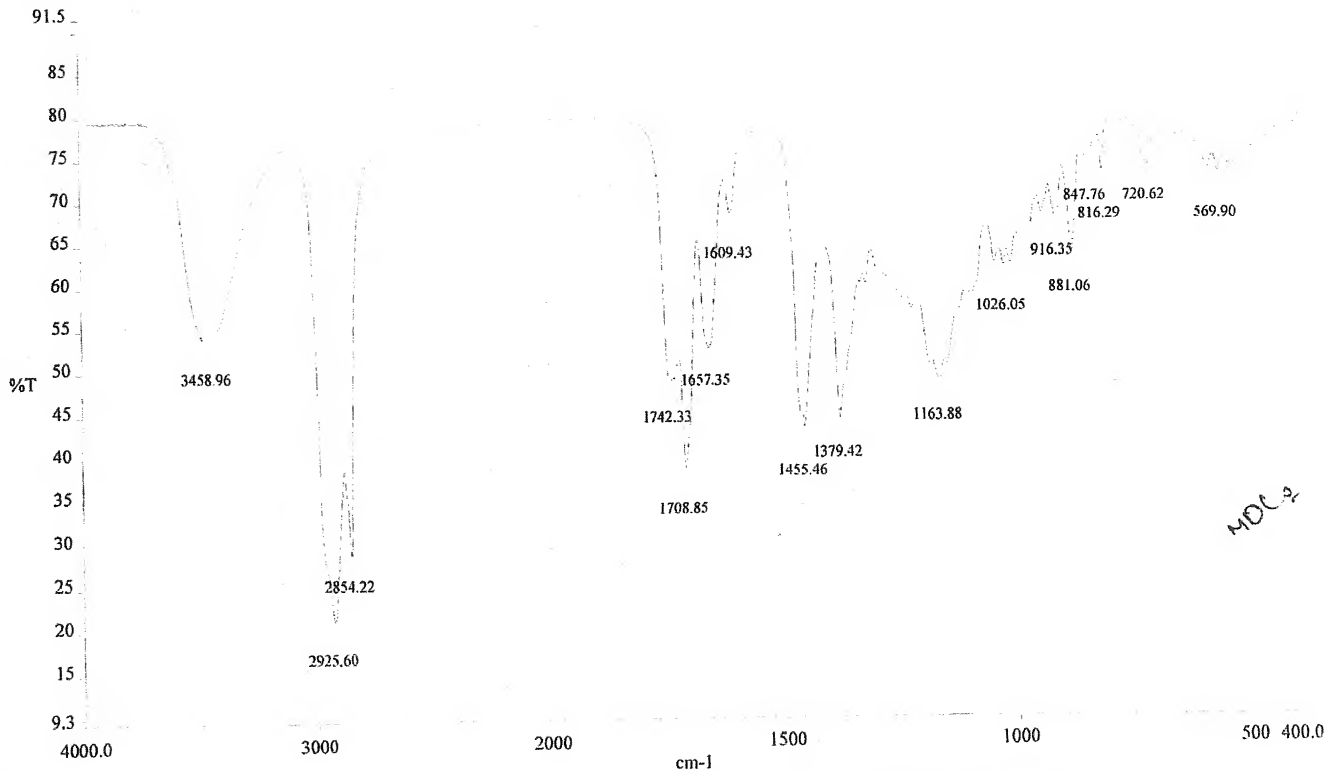
FTIR Spectrum

SIPRA LABS

Instrument Serial Number: 72425

Resolution: 4.00 cm-1

Date: 09-8-06



Spectrum Name: NP-1459-3.002

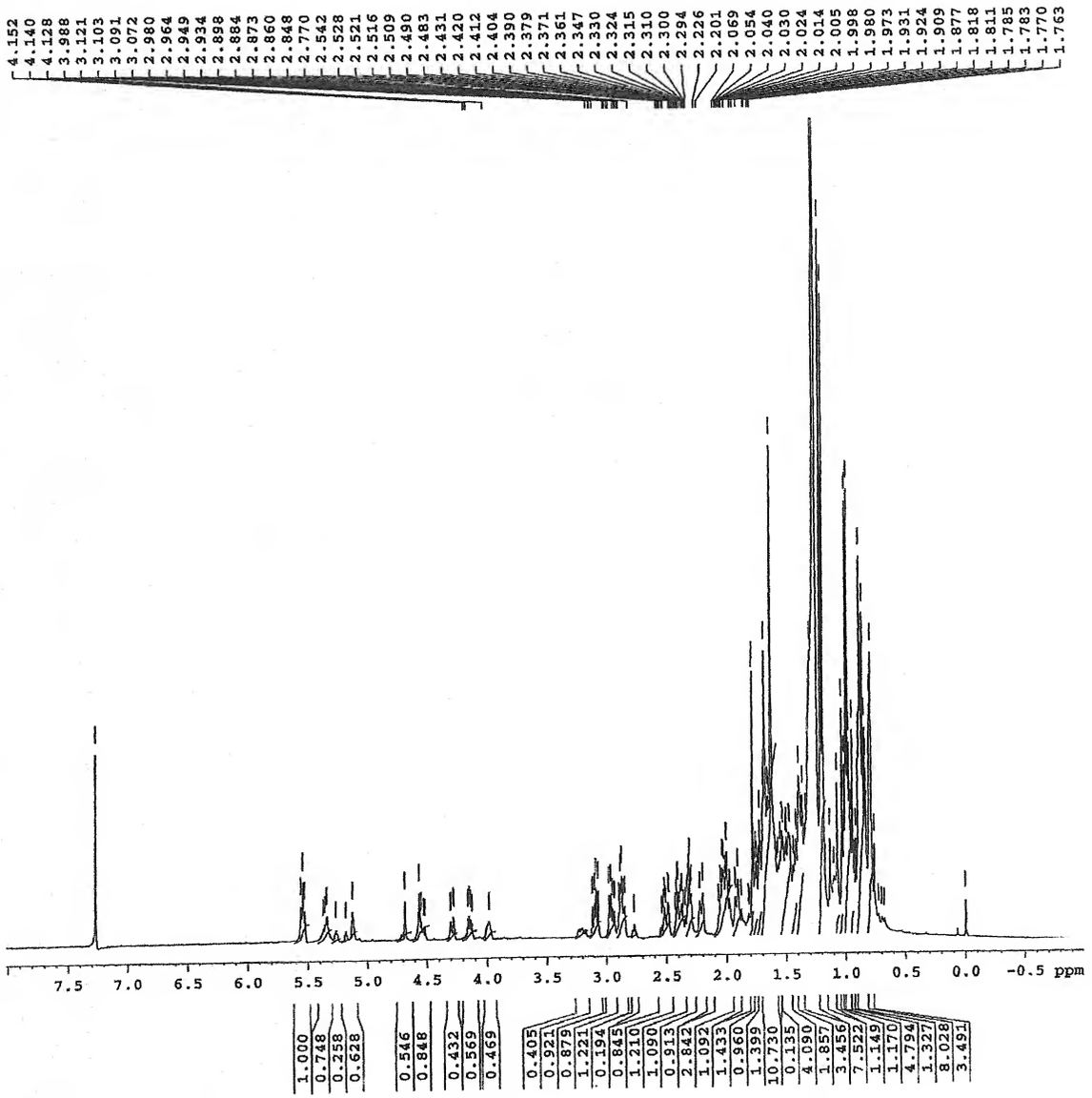
Description: EXTRACT B3,B3

Comments: KARNATAKA COLLEGE OF PHARMACY

Spectrum Pathname: C:\pel_data\spectra\NP-1459-3.002

MDC 9

Fig. No. 14



B 3

Current Data Parameters

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EXPNO	7
PROCNO	1

F2 - Acquisition Parameters

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Time	11.46
INSTRUM	spect
PROBHD	TXI Z8161/0179
PULPROG	zg
TD	16384
SOLVENT	CDCl3
NS	256
DS	0
SWH	6009.615 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	362
DW	83.200 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

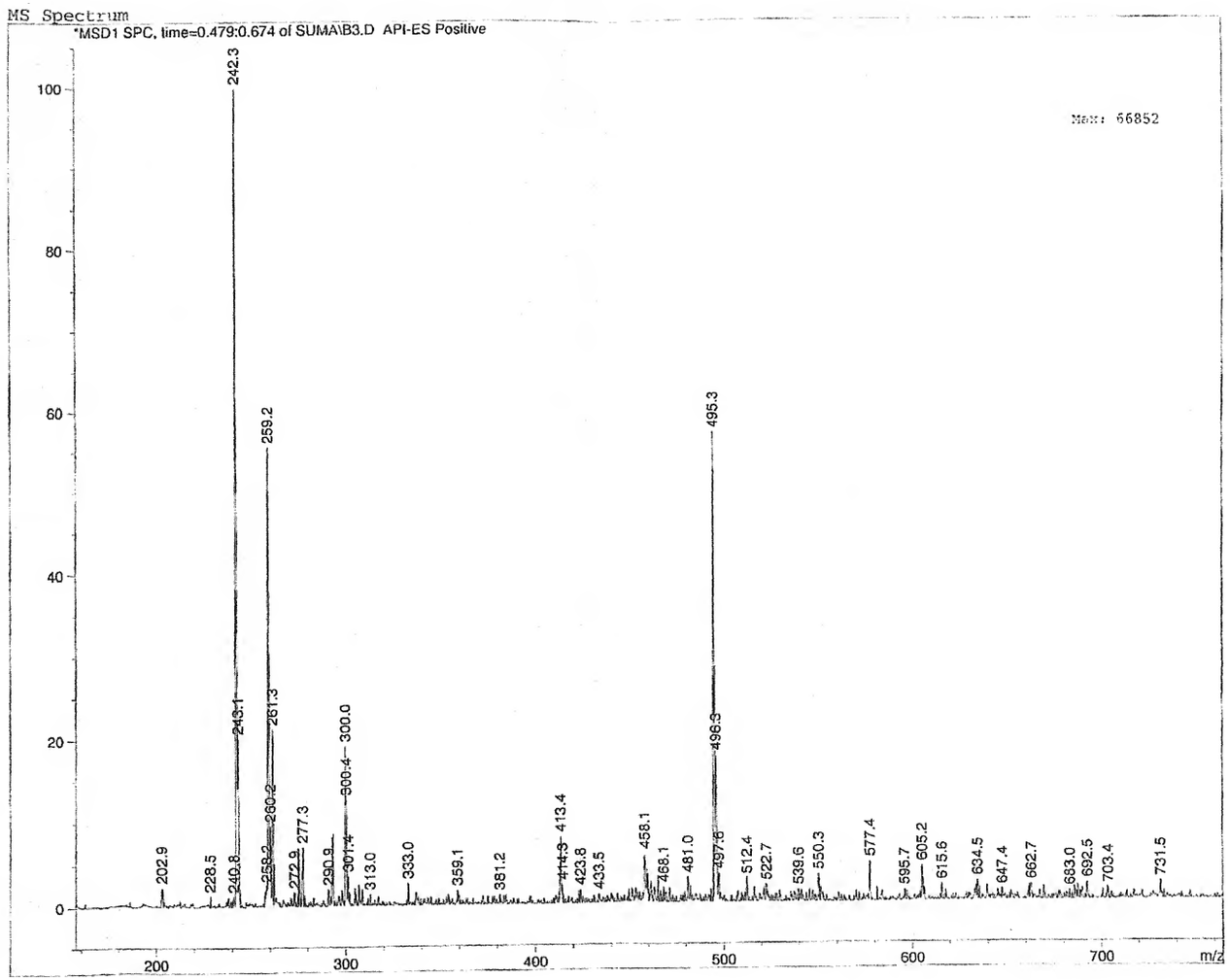
NUC1	1H
P1	9.50 usec
PL1	0.00 dB
SFO1	500.0319648 MHz

F2 - Processing parameters

SI	65536
SF	500.0300083 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

Fig. No. 15

of window 80: MS Spectrum



Instrument 1 9/26/06 10:33:38 AM suma

74

Fig. No. 16

NABL

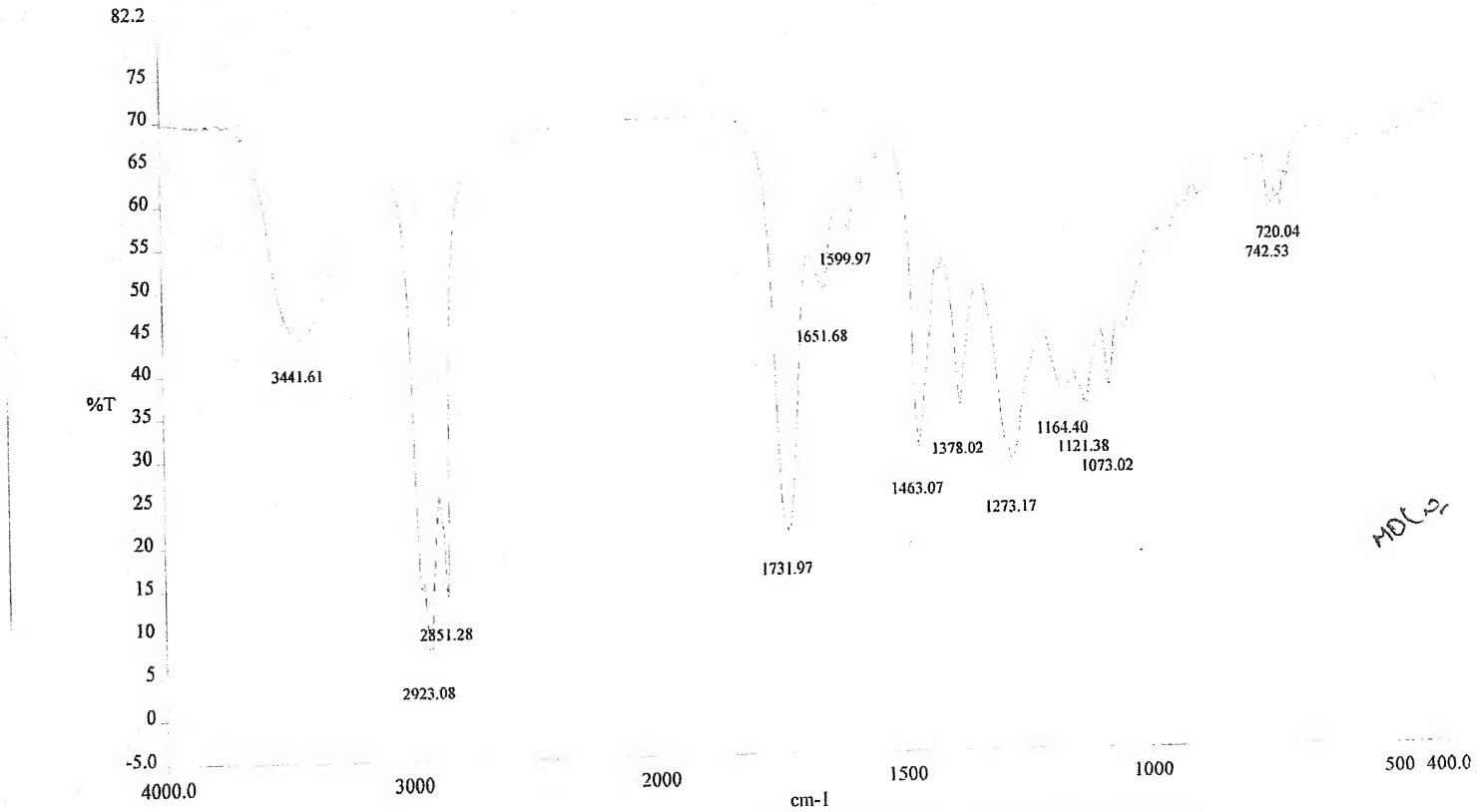
FTIR Spectrum

SIPRA LABS

Instrument Serial Number: 72425

Resolution: 4.00 cm-1

Date: 09-8-06



Spectrum Name: NP-1459-5.002

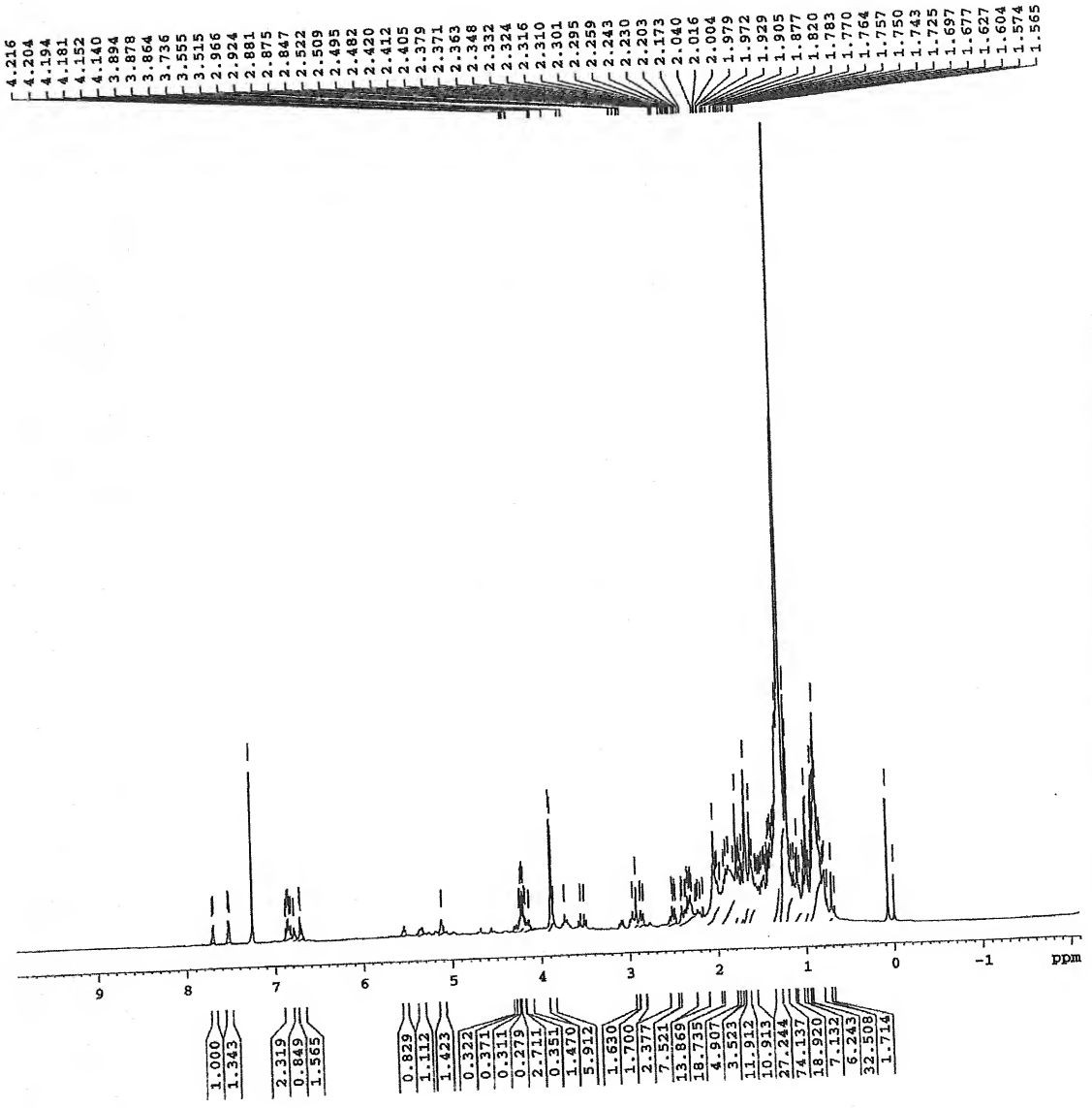
Description: EXTRACT B5,B5

Comments: KARNATAKA COLLEGE OF PHARMACY

Spectrum Pathname: C:\pel_data\spectra\NP-1459-5.002

MDC-2

Fig. No. 17



B 5

Current Data Parameters

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EXPNO	8
PROCNO	1

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Time	12.57
INSTRUM	spect
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PULPROG	zg
TD	16384
SOLVENT	CDCl3
NS	256
DS	0
SWH	6009.615 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	101.6
DW	83.200 usec
DE	6.00 usec
TE	298.1 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

NUC1	1H
P1	9.50 usec
PL1	0.00 dB
SFO1	500.0319648 MHz

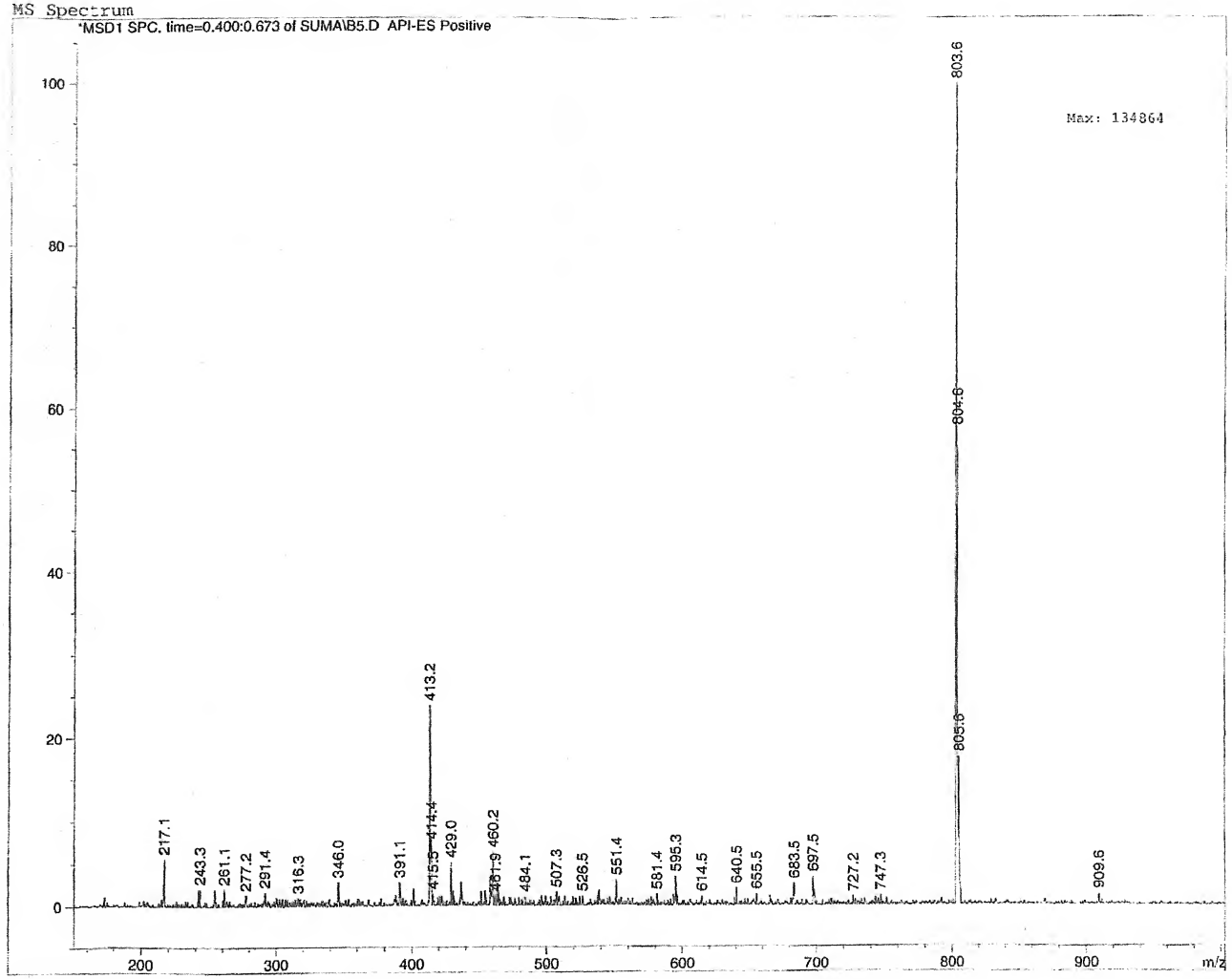
F2 - Processing parameters

SI	65536
SF	500.0300111 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

Fig. No. 18

nt of window 80: MS Spectrum

MS Spectrum



Instrument 1 9/26/06 10:50:57 AM suma

77

Fig. No. 19

NABL

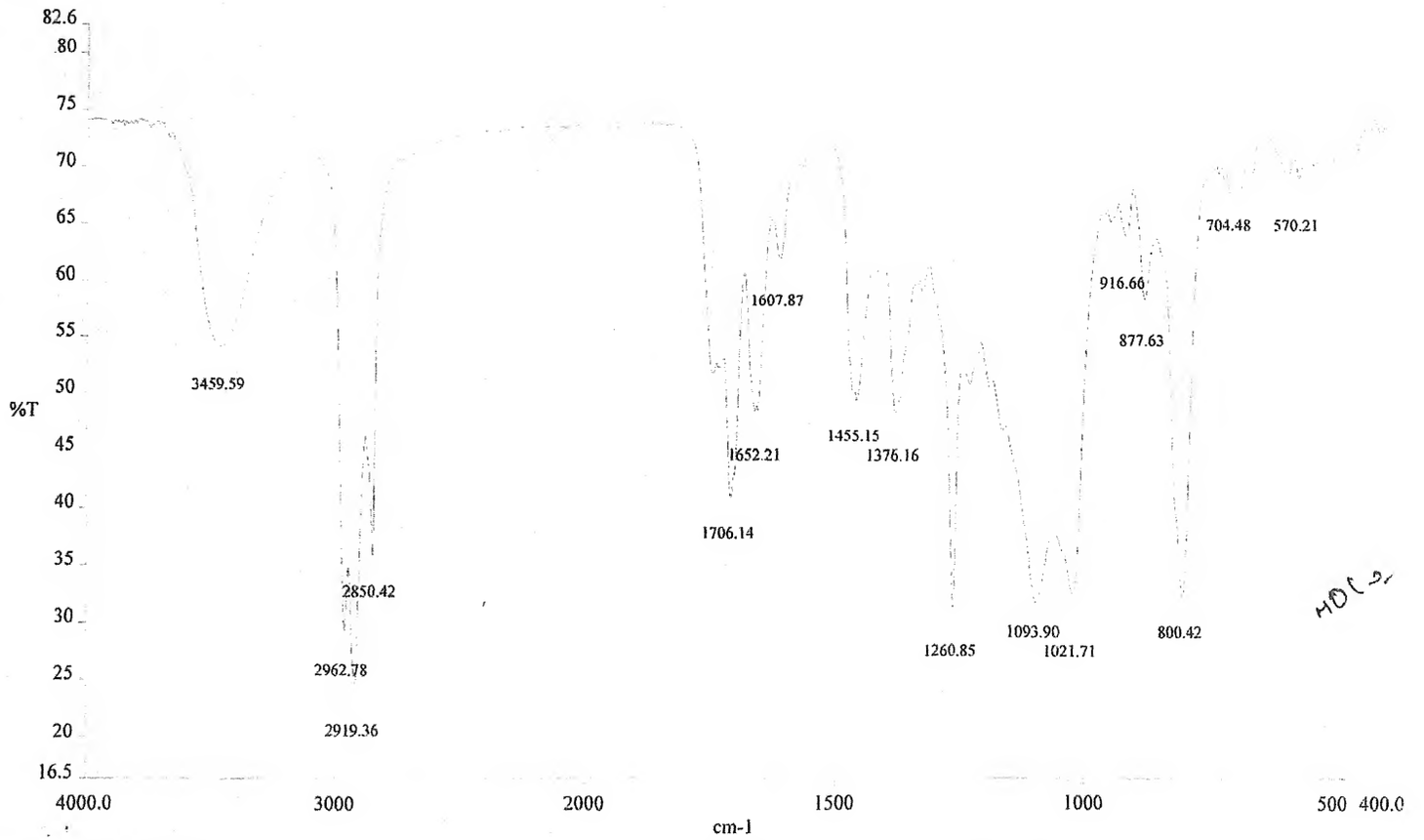
FTIR Spectrum

SIPRA LABS

Instrument Serial Number: 72425

Resolution: 4.00 cm-1

Date: 09-8-06



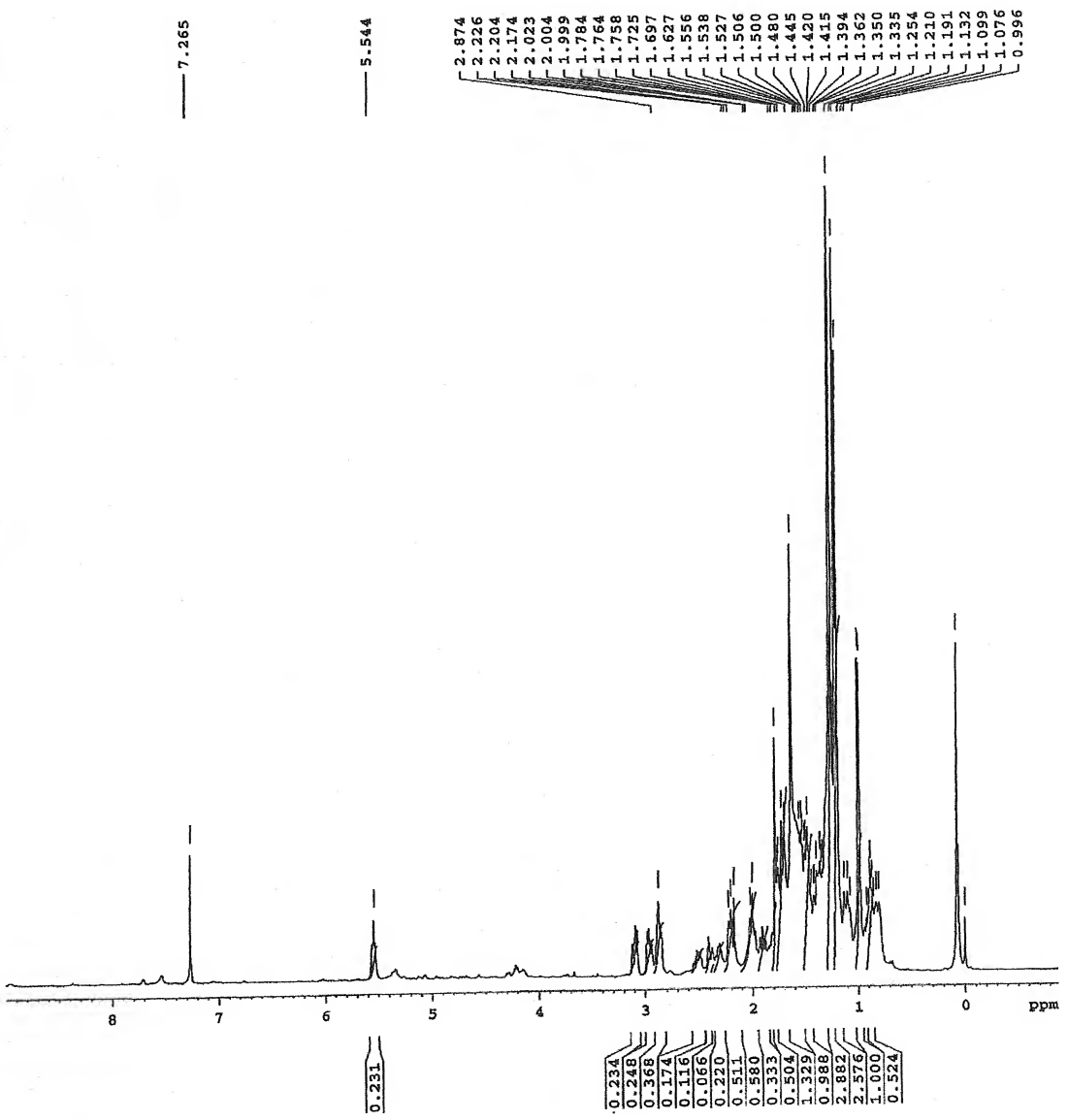
Spectrum Name: NP-1459-6.002

Description: EXTRACT,M1

Comments: KARNATAKA COLLEGE OF PHARMACY

Spectrum Pathname: C:\pel_data\spectra\NP-1459-6.002

Fig. No. 20



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Current Data Parameters
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EXPNO 1
PROCNO 1

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PULPROG	zg
TD	16384
SOLVENT	CDCl3
NS	128
DS	0
SWH	6009.615 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	71.8
DW	83.200 usec
DE	6.00 usec
TE	297.8 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

NUC1	1H
P1	9.52 usec
PL1	0.00 dB
SFO1	500.0325750 MHz

F2 - Processing parameters

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SF	500.0300084 MHz
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SSB	0
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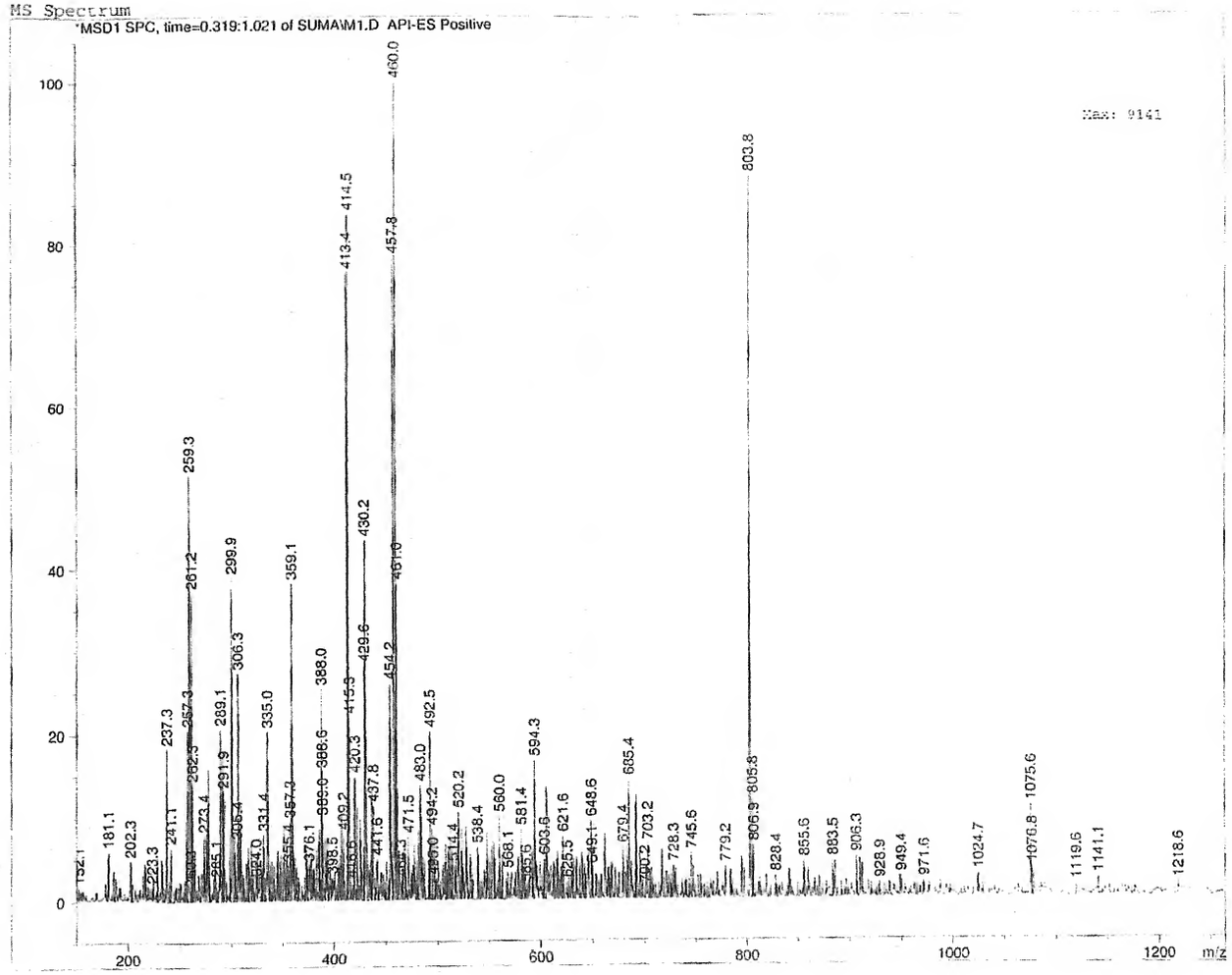
GB	0
PC	1.00

Fig. No. 21

MS Spectrum



80

Fig. No. 22

NABL

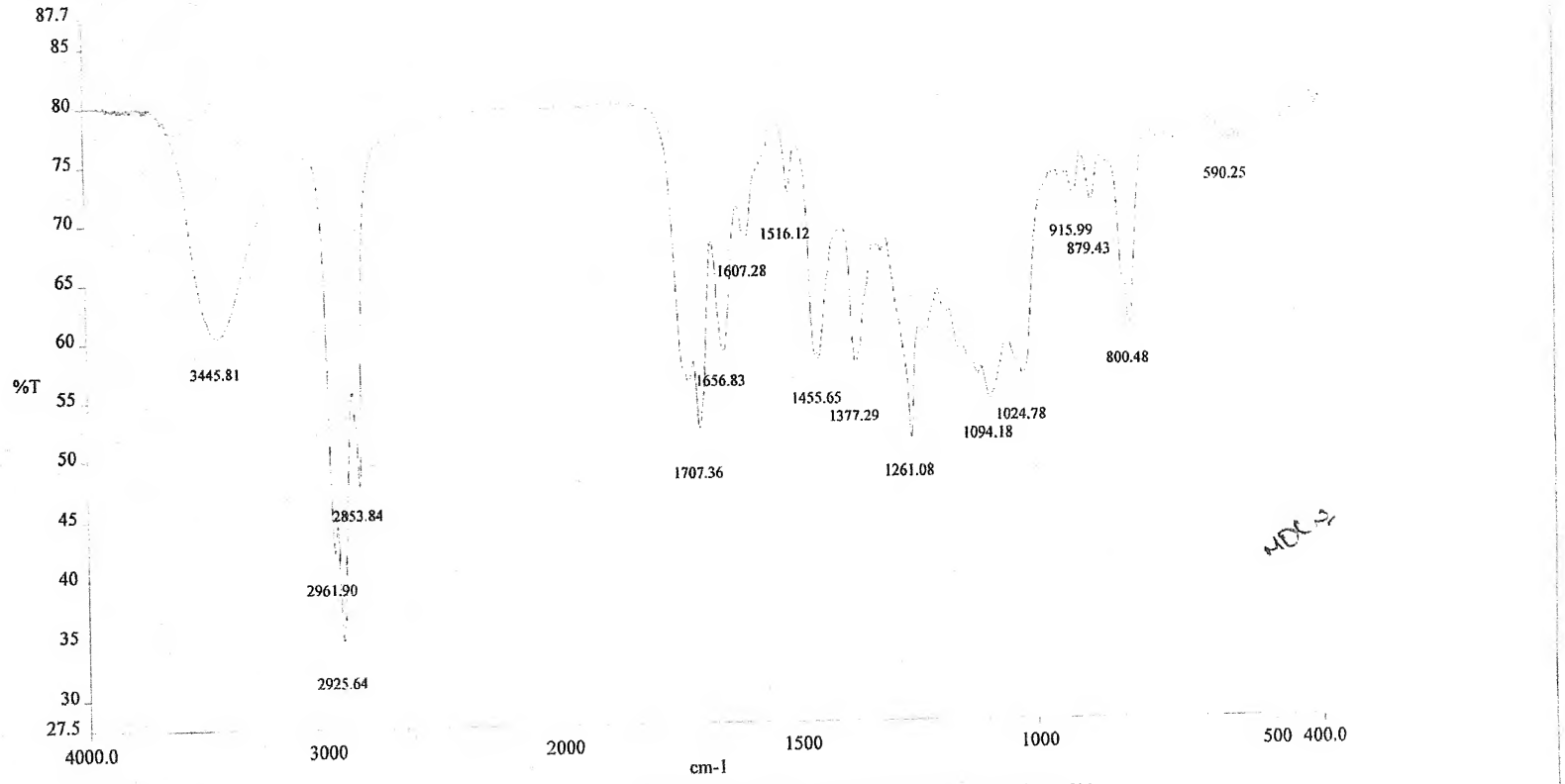
FTIR Spectrum

SIPRA LABS

Instrument Serial Number: 72425

Resolution: 4.00 cm-1

Date: 09-8-06



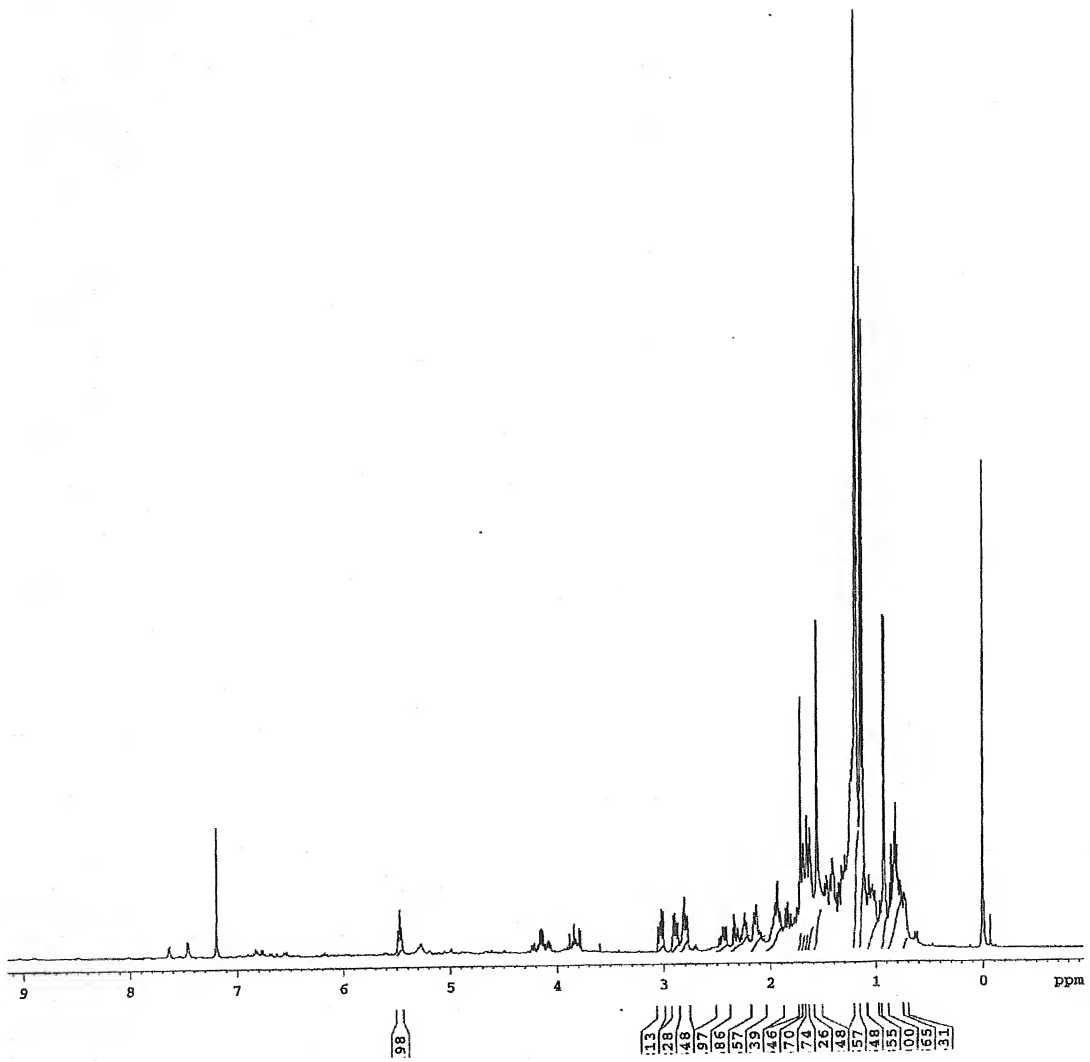
Spectrum Name: NP-1459-7.002

Description: EXTRACT,M2

Comments: KARNATAKA COLLEGE OF PHARMACY

Spectrum Pathname: C:\pel_data\spectra\NP-1459-7.002

Fig. No. 23



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Current Data Parameters
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EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
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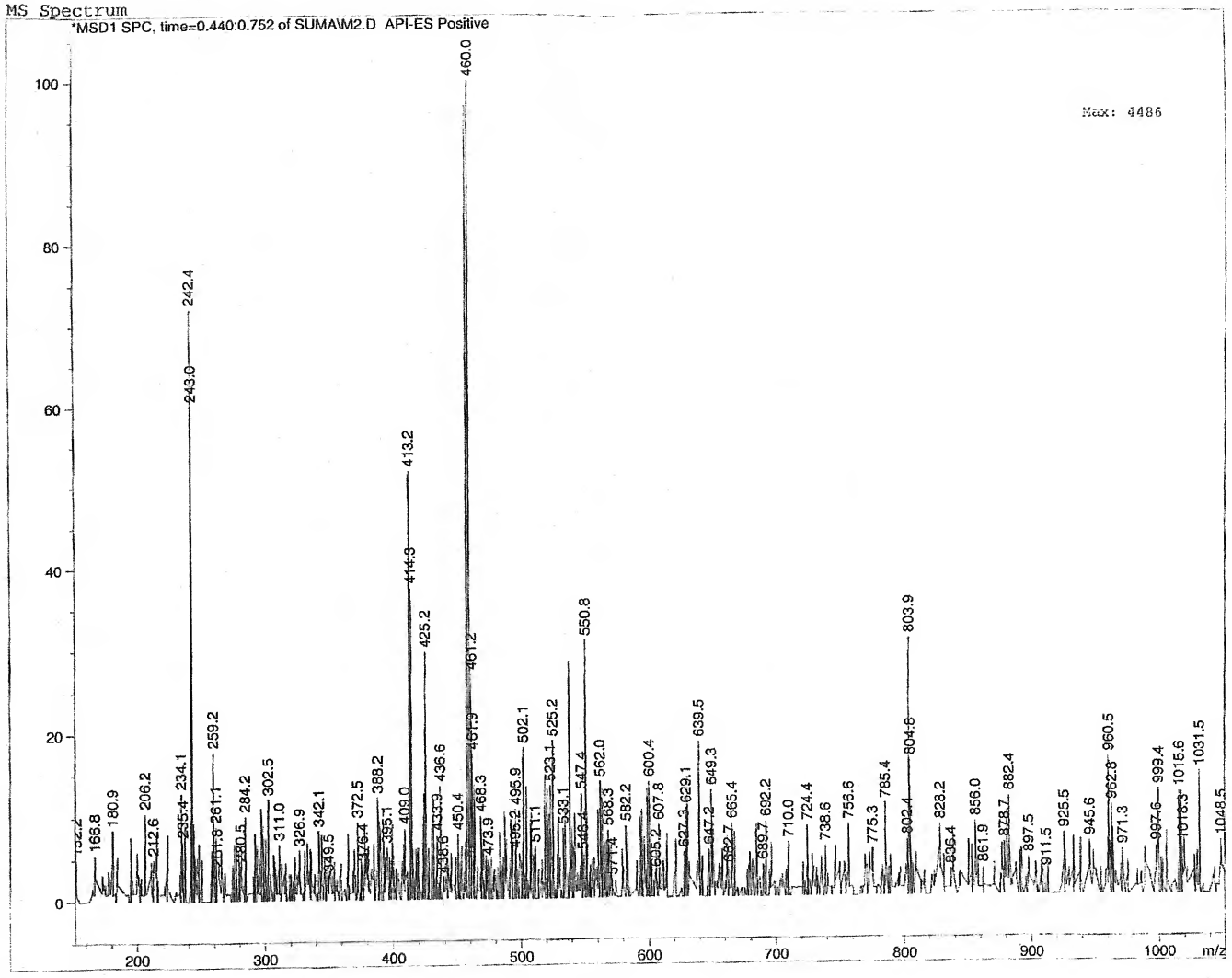
Time 6.12
INSTRUM spect
PROBHD 5 mm BBI Z-Gra
PULPROG zg
TD 16384
SOLVENT CDCl3
NS 128
DS 0
SWH 6009.615 Hz
FIDRES 0.366798 Hz
AQ 1.3631988 sec
RG 57
DW 83.200 usec
DE 6.00 usec
TE 297.8 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.52 usec
PL1 0.00 dB
SFO1 500.0325750 MHz

F2 - Processing parameters
SI 65536
SF 500.0300432 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

Fig. No. 24



Instrument 1 9/26/06 12:27:14 PM suma

Fig. No. 25

NABL

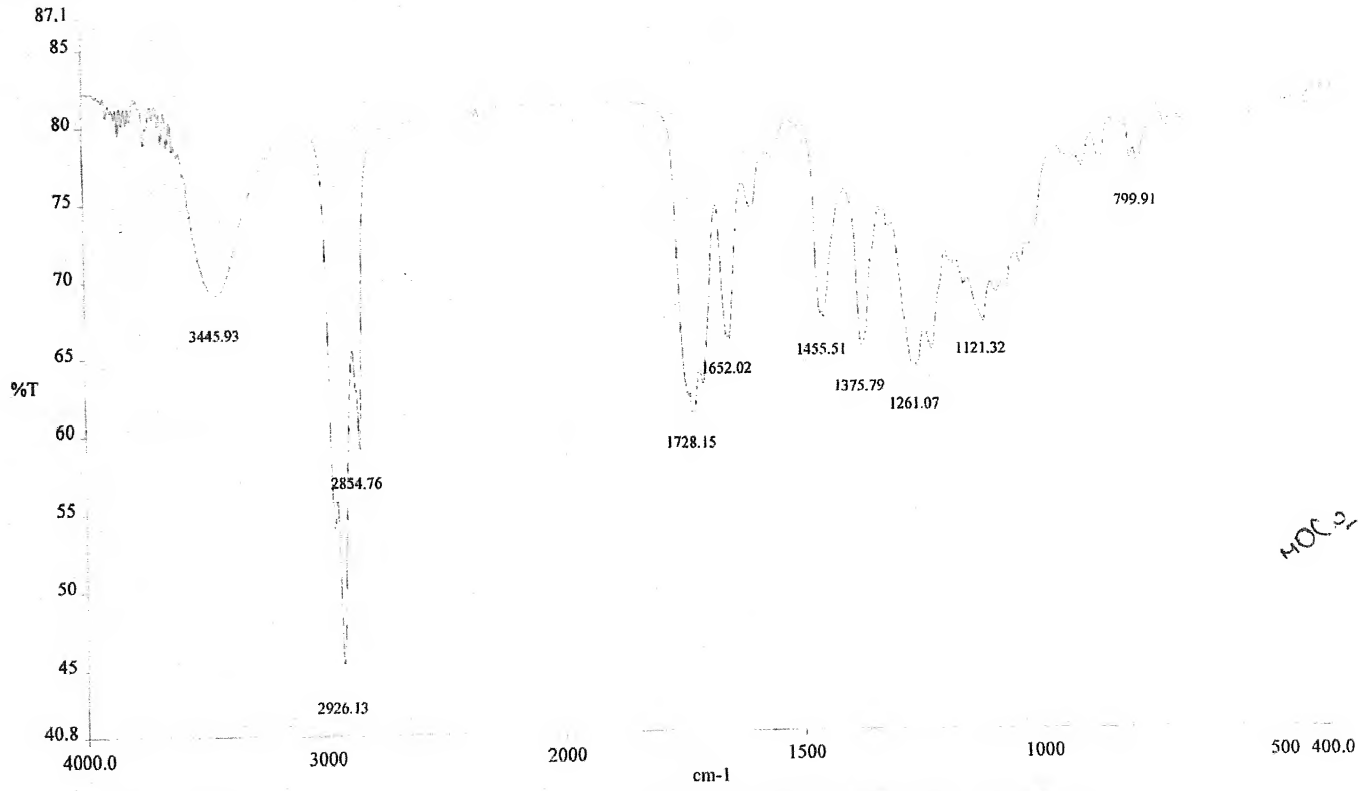
FTIR Spectrum

SIPRA LABS

Instrument Serial Number: 72425

Resolution: 4.00 cm-1

Date: 09-8-06



Spectrum Name: NP-1459-8.002

Description: EXTRACT,M3

Comments: KARNATAKA COLLEGE OF PHARMACY

Spectrum Pathname: C:\pel_data\spectra\NP-1459-8.002

Fig. No. 26

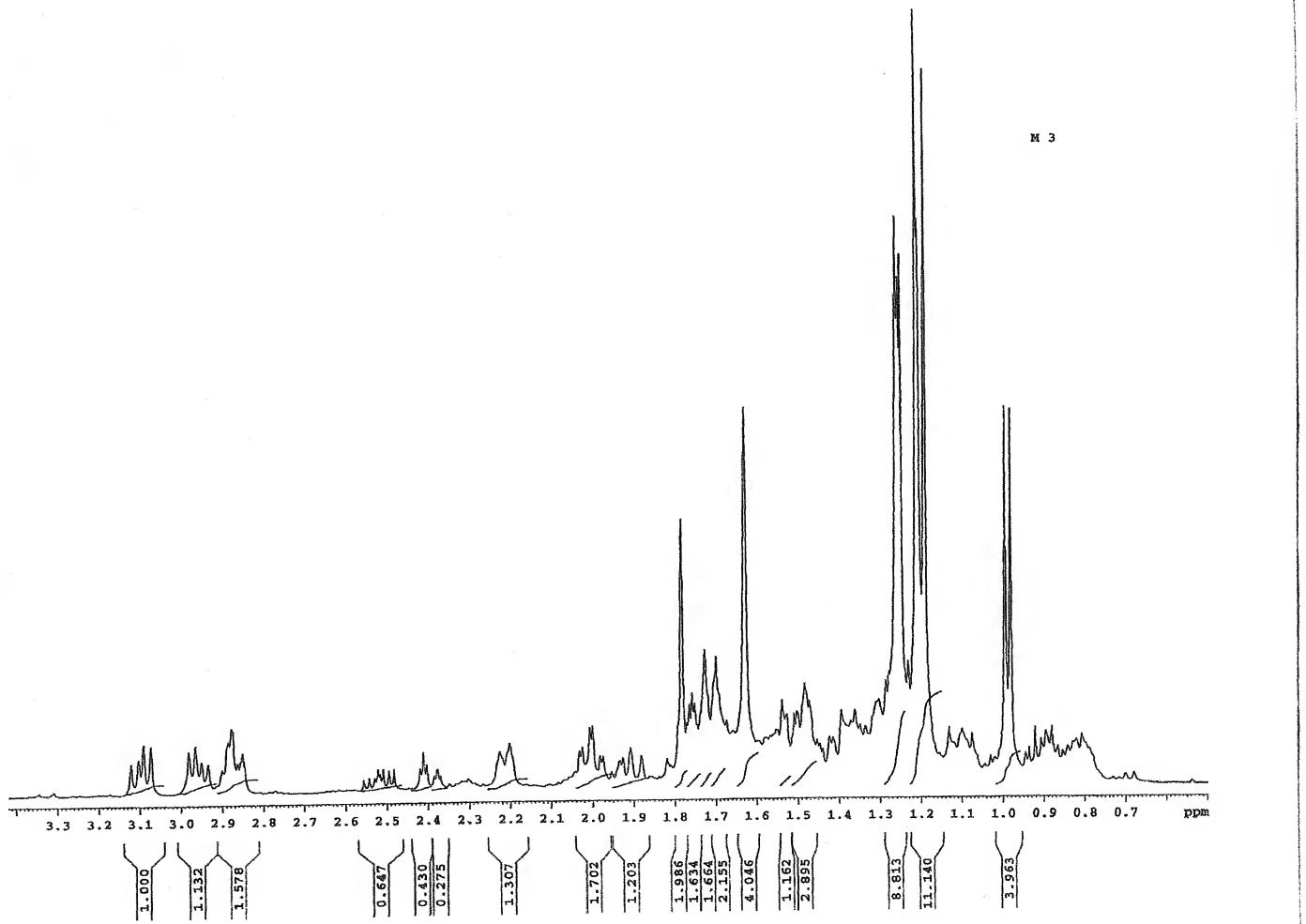
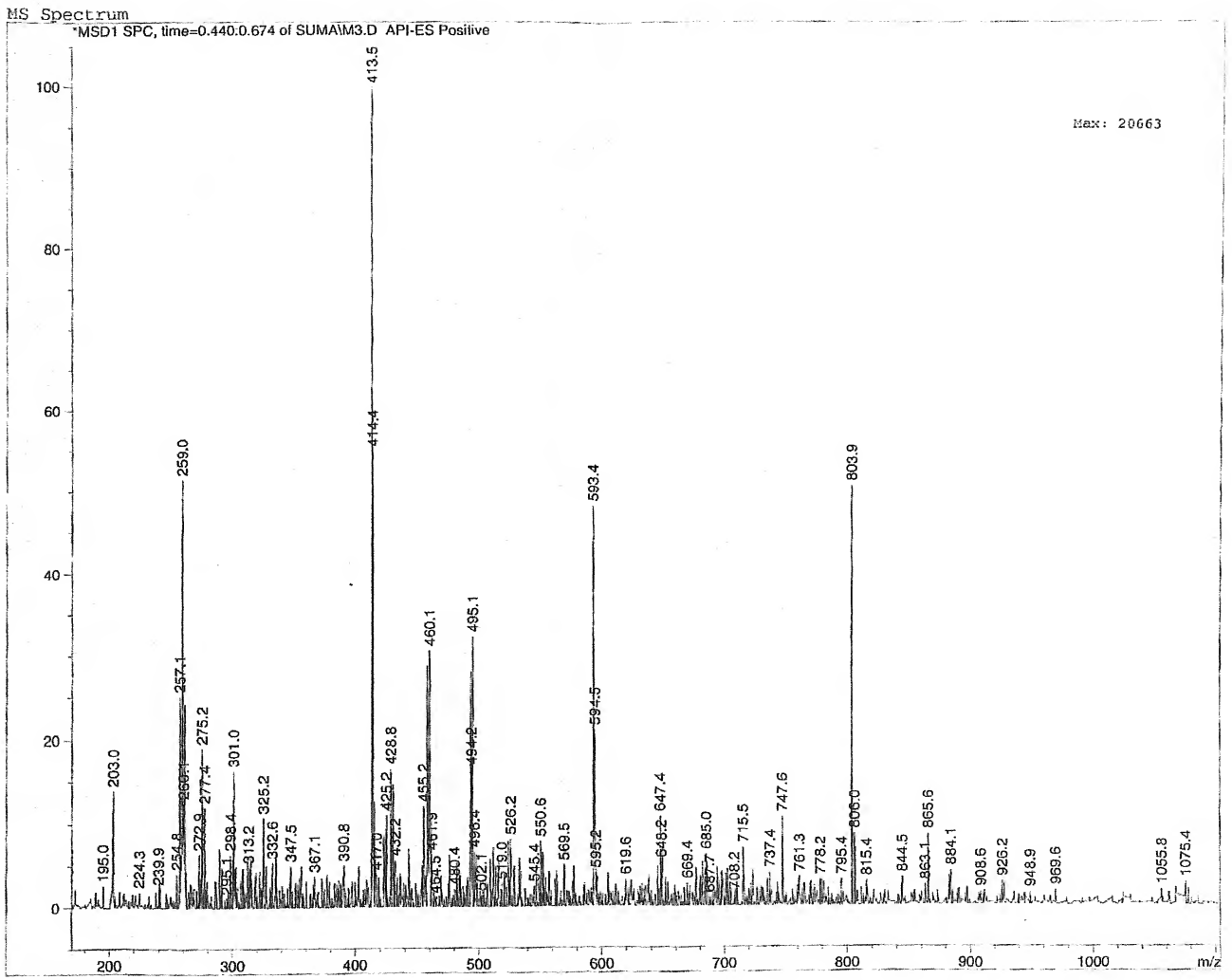

M 3
3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 0.7 ppm
1.000
1.132
1.578
0.647
0.430
0.275
1.307
1.702
1.203
1.986
1.634
1.664
2.155
4.046
1.162
2.895
8.813
11.140
3.963

Fig. No. 27

nt or window 80: MS Spectrum



Instrument 1 9/26/06 12:35:42 PM suma

Fig. No. 28

PHARMACOLOGICAL STUDY

PHARMACOLOGICAL STUDY

ANTIMICROBIAL ACTIVITY

Herbal medicine represents one of the most important fields of traditional medicine in India especially, in rural areas. Thus, phytotherapy is practiced by large proportion of Indian population for the treatment of several physical, physiological, mental, and social ailments. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study the medicinal plants, which have folklore reputation in a more intensified way.

Within the recent years, infections have increased to a great extent and antibiotics resistance becomes ever- increasing therapeutics problem (Austin et al ., 1999) . Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action (Hamil et., al 2003 ; Machado et al ., 2003 ; Motesi et al ., Barbour et al ; 2004) .

Medicinal plants are natural resources, yielding valuable herbal products which often used in the treatment of various diseases. During the last few years, probably due to the increasing development of drug resistance to human pathogenic organisms as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections (paddock and Wise, 1989; Mulligan et al ., 1933 ; Davies , 1994 ; Enne et al ., 2001 Marchese and Schito , 2001 ; Poole , 2001), antimicrobial properties have been reported more frequently in wide range of plant extracts and natural products in attempt to discover new chemical classes of antibiotics that could resolve these problem .

Carissa carandas is a well known plant in herbal medicine due to therapeutic efficacy of *Carissa carandas* plant which is used traditionally in the treatment of

eczema, skin diseases, scabies internally as anthalmentic, antipyretic, stomachic, diarrhoea, bitter, anorexia, in pittha and kapha.

Antibacterial activity:

In-vitro anti-microbial studies of petroleum ether, benzene, chloroform, ethyl acetate, methanol, extracts of the roots was performed as follows.

Petroleum ether, benzene, chloroform, ethyl acetate, and methanol were dissolved in dimethylsulphoxide (DMSO) to get 0.025 mg/ml, 0.05 mg/ml, 0.1mg/ml, 0.2 mg/ml, 0.4 mg/ml, 0.8 mg/ml, 1mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, and 32 mg/ml. The anti-microbial activity was studied by employing 24 hr culture of *E. coli, Bacillus subtilis and S. aureus* using nutrient agar medium. Microbial strains in the study were obtained from microbiology department of Gulbarga University Gulbarga. The medium was sterilized by autoclaving at 120 °C (15 lb /n^2). About 30 ml of molten medium inoculated with the strains of microbes were transformed aseptically in to each sterilized petriplates (10 cm diameter). The plates were kept at a room temperature to allow solidification in each plate well of 6mm diameter made using borer. Accurately 0.2 ml of test and standard solution were transferred to cups aseptically and labeled accordingly. The plates were then maintained at room temperature for two hours to allow diffusion of the solutions in medium. The Petri-dishes used for anti-microbial activity at 37 ^{0}C for 24 hrs. The diameter of zone of inhibition surrounds each of well was recorded.

Minimum inhibition concentration:

Benzene extract was taken to study the minimum inhibition concentration (MIC). The Table 2 showed that the benzene gave the MIC at 0.1 mg/ml against all tested microorganism. The result revealed that the title plant benzene root extract has exhibited action against *E. coli, Bacillus subtilis* and *S. aureus*.

Antifungal activity:

In vitro anti-fungal studies of petroleum ether ,benzene , chloroform , ethyl acetate, methanol, extracts of the roots was performed as follows. Petroleum ether , benzene, chloroform, ethyl acetate, and methanol were dissolved in dimethylsulphoxide (DMSO) to get 0.025 mg /ml, 0.05 mg /ml, 0.1mg /ml, 0.2 mg /ml, 0.4 mg /ml, 0.8 mg /ml, 1 mg /ml, 2 mg /ml, 4 mg /ml, 8 mg /ml, 16 mg /ml, and 32 mg /ml.

The anti fungal activity was studied by employing 24 hours culture of A . *Niger, A. Fumigatus* using potato agar medium .The fungal strains in the study were obtained from microbiology department of Gulbarga University Gulbarga. The medium was sterilized by autoclaving at 120 °C (15 lb /n^2). About 30 ml of molten medium inoculated with the strains of fungi were transformed aseptically in to each sterilized petriplates (10 cm diameter). The plates were kept at a room temperature to allow solidification in each plate well of 6mm diameter made using borer. Accurately 0.2 ml of test and standard solution were transferred to cups aseptically and labeled accordingly.

The plates were then maintained at room temperature for two hours to allow diffusion of the solutions in medium. The Petri dishes used for anti fungal activity at 28^0c for 24 hours. The diameter of zone of inhibition surrounds each of well was recorded.

Minimum inhibition concentration:

Benzene extract was taken to study the minimum inhibition concentration (MIC). The Table 2 showed that the benzene gave the MIC at 0.2 mg/ml against all tested against fungal strains. The (Table 2) also showed that the methanolic extract gave the MIC at

1.6 mg/ml against all tested against fungal strains. The result revealed that the title plant benzene root extract has exhibited action against *A. Niger and A. fumigatus*.

Statistical Evaluation:

The results are expressed as mean ± standard error of mean (S.E .M). The results obtained from this study were evaluated by students' t' test doses were compared with control group and ulcer index was calculated along with scoring the severity of ulcer.

RESULTS

Antibacterial activity:

As shown in table 4 the MIC of benzene extract is too low and have an efficiency to produce at 0.1 mg/ml. The streptomycin treatment shown similar antimicrobial results as reported by earlier studies. The zone of inhibition was 15 ± 0.2978, 10 ± 0.258 and 12 ± 0.2106 and percent inhibition was 83.33, 62.5 and 85.71 respectively against *E. coli, Bacillus subtilis* and *Streptococcus aureus.* Linear, concentration dependent antimicrobial zone of inhibition and percent inhibition better than streptomycin in crude form against all types of microbials selected in the present study was demonstrated in benzene extract. The percent inhibition was almost 90 percent against all the species considered for the study. Statistical analysis of the results revealed that Benzene extract even at lower dose 1 mg/ml has significant ($P<0.05$) antimicrobial property. However, other extracts failed to reproduce the same results (Table 5).

Antifungal activity:

As depicted in table 6 & 7, the MIC of benzene extract is too low and have an efficiency to produce at 0.2 mg/ml, when compared to methanolic extract which possess MIC at 1.6 mg/ml. The streptomycin treatment shown similar antifungal results as reported by earlier studies. Similar to antibacterial activity benzene extract has shown significant ($P<0.05$) zone of inhibition at 4 mg/ml. Whereas, methanolic

extract has shown the significant ($P<0.05$) at 8 mg/ml against both the fungal species selected in the present study. The percent inhibition of benzene extract at maximum dose was 90 and 85 percent against *A. fumigatus* and *A. niger* respectively. The methanolic extract has produced 70 and 75 percent zone inhibitory effect against these two species respectively (Table 8).

DISCUSSION

In the present study benzene extract has shown significant antibacterial and antifungal activities. These activities possibly, due to the presence of saturated, unsaturated fatty acids and aromatic amides as shown in spectral studies. The antifungal activity of the methanolic extract may be due to aromatic amides.

The previous several significant studies have shown that the saturated fatty acids, fatty amines, unsaturated fatty acids are effective even against methicillin resistant *staphylococcus aureus* by involving multichannel and oxygen derivative mechanisms (Alexander, et al., 2003; Takashi, et al., 2004; Kabara, 1979; Kabara et al., 1977; Kabara et al., 1972; Olitzky, 1962). It is also being proposed that the fatty acid conjugation with peptides results in an increased ability to permeabilise bacteria membranes corresponding to an increased helical structure in the presence of phospholipid vesicles (Kabara et al., 1972). The increased antimicrobial activity of fatty acids also depends on length of fatty acids with number of unsaturated bonds in general with an ability of esterification of the carboxyl group (Kabara et al., 1977). The antimicrobial activity of the fatty acid contents of benzene extract may be one of the above mechanism or all of them.

In contrary, the mechanism of amides will not depend on chain length and number of double or triple bonds (Alexander et al., 2003). It has been hypothesized to produce its effects through conjugation with peptides and proteins of surface membranes

thereby increase the permeability of the membrane and effective against all types of microbes (Takashi, et al., 2004; Kabara, 1979). This could be the reason behind the antifungal activity of methanolic extract as the phytochemical investigation and spectral analysis has shown the presence of aromatic amides. The aromatic amide was also found in the benzene extract, which could be the reason for its effectiveness as antibacterial and antifungal agent.

We conclude from the present study that, the benzene extract contains both fatty acids and fatty amides thereby producing its significant antimicrobial properties irrespective of gram positive and gram-negative species may be due to synergistic combinations. However, further studies are required to find out possibility of the isolated compound's antimicrobial properties individually and in combination after the purification.

TABLE 4

DETERMINATION OF MINIMUM INHIBITION CONCENTRATION OF BENZENE EXTRACT BY CUP PLATE METHOD

Species	Streptomycin	Control	F1 0.025mg/ml	F2 0.05mg/ml	F3 0.1mg /ml	F4 0.2mg/ml	F5 0.4 mg /ml	F6 0.8mg /ml
Escherechia coli	15 mm	-	-	-	5 mm	6 mm	10 mm	11 mm
Bacillus subtilis	6 mm	-	-	-	5 mm	7 mm	7.5 mm	8 mm
Streptococcus aureus	5 mm	-	-	-	2 mm	3 mm	4 mm	5 mm

The above tabular column shows that the minimum inhibition concentration of benzene extract is 0.1mg/ml

TABLE 5

ANTIBACTERIAL ACTIVITY OF *CARISSA CARANDAS LINN* ROOT EXTRACT BY CUP PLATE METHOD

Treatment	*E. Coli*		*Bacillus Subtilis*		*S. Aurius*	
	Zone of Inhibition (mm)	% Inhibition of radial growth	Zone of Inhibition (mm)	% Inhibition of radial growth	Zone of Inhibition (mm)	% Inhibition of radial growth
5% DMSO	00	00	00	00	00	00
Streptomycin	15 ± 0.2978	83.33	10 ± 0.258	62.5	12 ± 0.2106	85.71
PE 1 mg/ml	2 ± 0.233	11.11	00	00	2 ± 0.233	14.28
PE 2 mg/ml	4 ± 0.3956	22.22	00	00	3 ± 0.3785	21.42
PE 4 mg/ml	5 ± 0.4458	27.77	00	00	4 ± 0.4711	28.57
PE 8 mg/ml	6 ± 0.2106	33.33	00	00	5 ± 0.2106	35.71
PE 16 mg/ml	6 ± 0.258	33.33	00	00	5 ± 0.258	35.71
PE 32 mg/ml	7 ± 0.4711	38.89	00	00	6 ± 0.258*	42.85
BE 1 mg/ml	10 ± 0.2581*	55.55	8 ± 0.258*	50	9 ± 0.149**	64.28
BE 2 mg/ml	11± 0.258 **	61.11	9 ± 0.106 *	56.25	10 ± 0.2581**	71.42
BE 4 mg/ml	12 ± 0.5374**	66.67	11 ± 0.2106**	68.75	10 ± 0.2106**	71.42
BE 8 mg/ml	13 ± 0.4163**	72.22	12 ± 0.4471**	75	11 ± 0.4163***	78.57
BE 16 mg/ml	15 ± 0.4714***	83.33	13 ± 0.378***	81.25	12 ± 0.2106***	85.71
BE 32 mg/ml	16 ± 0.4942***	88.89	15 ± 0.258***	93.75	13 ± 0.2581***	92.85
CE 1 mg/ml	00	00	00	00	00	00
CE 2 mg/ml	00	00	00	00	00	00
CE 4 mg/ml	1 ± 00	5.55	00	00	1 ± 00	7.14
CE 8 mg/ml	1 ± 0.2106	5.55	1 ± 0.1489	6.25	2 ± 0.2106	14.28
CE 16 mg/ml	1 ± 0.2106	5.55	1 ± 0.1489	6.25	2 ± 0.2106	14.28
CE 32 mg/ml	2 ± 0.2106	11.11	1 ± 0.148	6.25	2 ± 0.2766	14.28
EAE 1 mg/ml	00	00	00	00	00	00
EAE 2 mg/ml	00	00	00	00	00	00
EAE 4 mg/ml	1 ± 00	5.55	00	00	00	00
EAE 8 mg/ml	1 ± 00	5.55	00	00	00	00
EAE 16 mg/ml	2 ± 0.2981	11.11	00	00	00	00
EAE 32 mg/ml	2 ± 0.2106	11.11	00	00	00	00
ME 1 mg/ml	1 ± 0.2581	5.55	00	00	00	00
ME 2 mg/ml	2 ± 0.2581	11.11	00	00	00	00
ME 4 mg/ml	2 ± 0.2981	11.11	00	00	00	00
ME 8 mg/ml	3 ± 0.5672	16.66	00	00	00	00
ME 16 mg/ml	3 ± 0.149	16.66	00	00	00	00
ME 32 mg/ml	4 ± 0.3651	22.22	00	00	00	00

TABLE 6

DETERMINATION OF MINIMUM INHIBITION CONCENTRATION OF BENZENE EXTRACT BY CUP PLATE METHOD AGAINST FUNGAL SPECIES.

Species	Fluconazole	Control	F1 0.025mg/ml	F2 0.05mg/ml	F3 0.1mg /ml	F4 0.2mg/ml
A. Fumigatus	10 mm	-	-	-	-	2 mm
A. Niger	12 mm	-	-	-	-	2 mm

TABLE 7

DETERMINATION OF MINIMUM INHIBITION CONCENTRATION OF METHANOLIC EXTRACT BY CUP plate method against fungal species.

Species	Fluconazole 1mg/ml	Control	F1 0.025mg/ml	F2 0.05mg/ml	F3 0.1mg /ml	F4 0.2mg/ml	F5 0.4 mg /ml	F6 0.8mg /ml	F6 1.6 mg /ml
A. Fumigatus	10 mm	-	-	-	-	-	-	-	2 mm
A. Niger	12 mm	-	-	-	-	-	-	-	2 mm

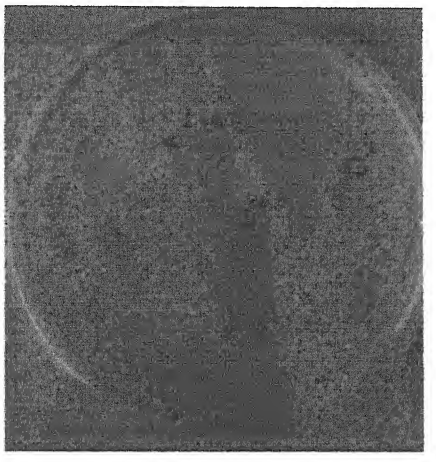
The above tabular column shows that the minimum inhibition concentration of benzene extract is 0.2 mg/ml and methanolic extract is 1.6 mg/ml.

TABLE 8
ANTIFUNGAL ACTIVITY OF *CARISSA CARANDAS* ROOT EXTRACT BY CUP PLATE METHOD

Treatment	*A. Fumigatus*		*A. Niger*	
	Zone of Inhibition (mm)	% Inhibition of radial growth	Zone of Inhibition (mm)	% Inhibition of radial growth
5% DMSO	00	00	00	00
Streptomycin	10 ± 0.258*	50	12 ± 0.666**	60
PE 1 mg/ml	--	--	--	--
PE 2 mg/ml	--	--	--	--
PE 4 mg/ml	--	--	--	--
PE 8 mg/ml	2 ± 0.148	10	1 ± 0.152	5
PE 16 mg/ml	3 ± 0.179	15	2 ± 0.148	10
PE 32 mg/ml	4 ± 0.25	20	3 ± 0.21	15
BE 1 mg/ml	4 ± 0.276	20	4 ± 0.21	20
BE 2 mg/ml	6 ± 0.21	30	5 ± 0.21	25
BE 4 mg/ml	9 ± 0.21*	55	8 ± 0.21*	40
BE 8 mg/ml	11 ± 0.258*	45	10 ± 0.333*	50
BE 16 mg/ml	13 ± 0.258**	65	12 ± 0.21**	60
BE 32 mg/ml	18 ± 0.666***	90	17 ± 0.666***	85
CE 1 mg/ml	4 ± 0.21	20	4 ± 0.21	20
CE 2 mg/ml	5 ± 0.21	25	6 ± 0.333	30
CE 4 mg/ml	7 ± 0.258	35	10 ± 0.21*	50
CE 8 mg/ml	8 ± 0.258*	40	12 ± 0.21**	60
CE 16 mg/ml	10 ± 0.21*	50	12 ± 0.258**	60
CE 32 mg/ml	12 ± 0.258**	60	13 ± 0.21**	65
EAE 1 mg/ml	--	-	--	--
EAE 2 mg/ml	4 ± 0.21	20	3 ± 0.21	15
EAE 4 mg/ml	6 ± 0.258	30	6 ± 0.258	30
EAE 8 mg/ml	8 ± 0.447*	40	8 ± 0.21*	40
EAE 16 mg/ml	9 ± 0.21*	45	12 ± 0.266**	60
EAE 32 mg/ml	12 ± 0.258**	60	13 ± 0.266**	65
ME 1 mg/ml	--	-	--	--
ME 2 mg/ml	2 ± 0.148	10	2 ± 0.148	10
ME 4 mg/ml	3 ± 0.217	15	4 ± 0.364	20
ME 8 mg/ml	9 ± 0.21*	45	10 ± 0.421*	50
ME 16 mg/ml	11 ± 0.21**	65	13 ± 0.21**	65
ME 32 mg/ml	14 ± 0.258**	70	15 ± 0.458**	75

FIG 29

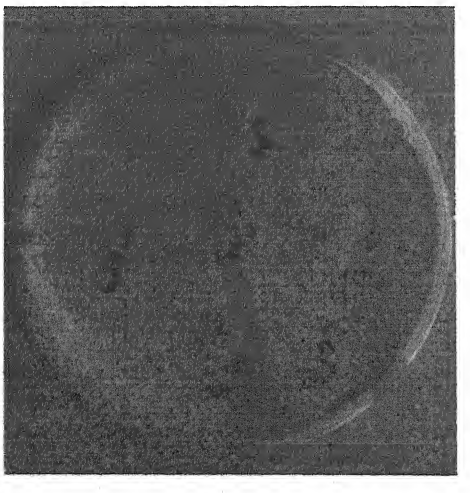
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [2 mg/ml] AGAINST E. COLI



[A2; PETROLEUM ETHER EXTRACT, B2; BENZENE EXTRACT, C2; CHLOROFOM EXTRACT, D2; ETHYL ACETATE EXTRACT, E2; METHANOLIC EXTRACTS]

FIG 30

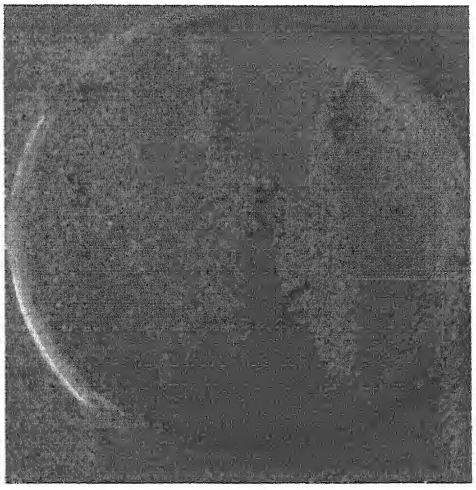
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [4 mg/ml] AGAINST E. COLI



[A3; PETROLEUM ETHER EXTRACT, B3; BENZENE EXTRACT, C3; CHLOROFOM EXTRACT, D3; ETHYL ACETATE EXTRACT, E3; METHANOLIC EXTRACTS]

FIG 31

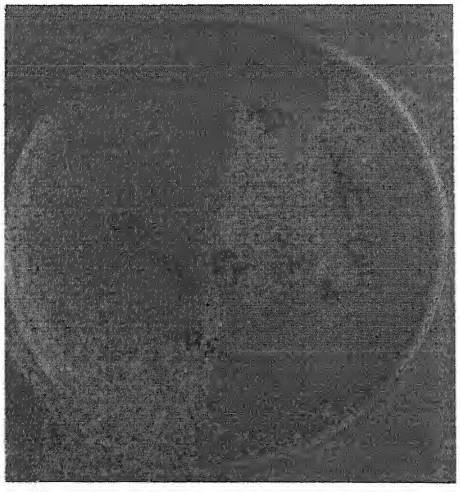
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [8 mg/ml] AGAINST E. COLI



[A4; PETROLEUM ETHER EXTRACT, B4; BENZENE EXTRACT, C4; CHLOROFOM EXTRACT, D4; ETHYL ACETATE EXTRACT, E4; METHANOLIC EXTRACTS]

IG 32

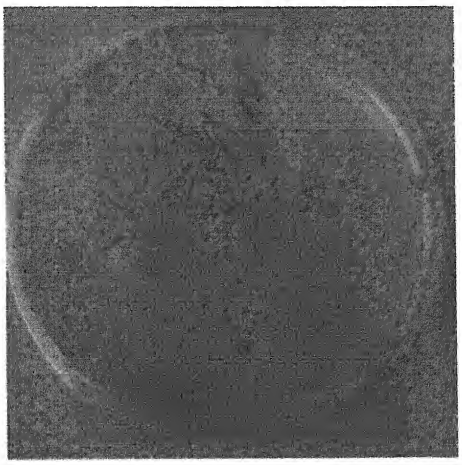
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [16 mg/ml] AGAINST E. COLI



[A3; PETROLEUM ETHER EXTRACT, B3; BENZENE EXTRACT, C3; CHLOROFOM EXTRACT, D3; ETHYL ACETATE EXTRACT, E3; METHANOLIC EXTRACTS]

FIG 33

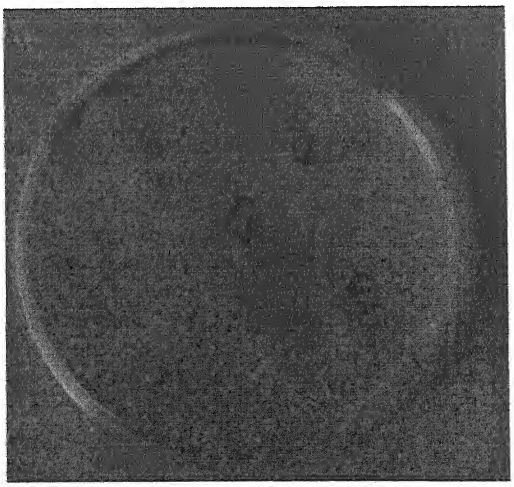
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [8 mg/ml] AGAINST B. SUBTILIS



[A2; PETROLEUM ETHER EXTRACT, B2; BENZENE EXTRACT, C2; CHLOROFOM EXTRACT, D2; ETHYL ACETATE EXTRACT, E2; METHANOLIC EXTRACTS]

FIG 34

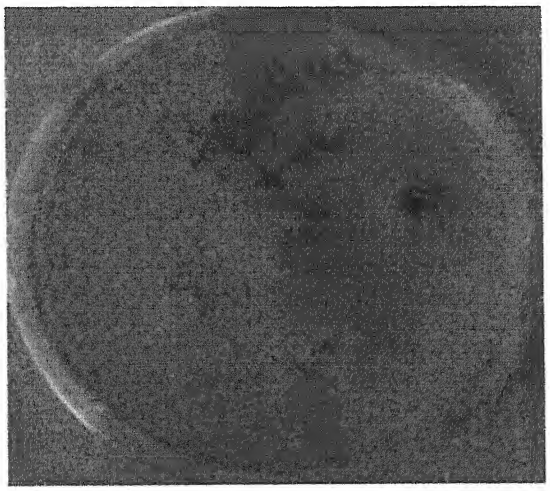
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [8 mg/ml] AGAINST B. SUBTILIS



[A3; PETROLEUM ETHER EXTRACT, B3; BENZENE EXTRACT, C3; CHLOROFOM EXTRACT, D3; ETHYL ACETATE EXTRACT, E3; METHANOLIC EXTRACTS]

FIG 35

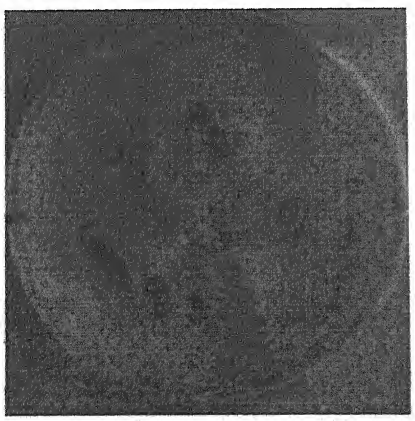
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [8 mg/ml] AGAINST B. SUBTILIS



[A5; PETROLEUM ETHER EXTRACT, B5; BENZENE EXTRACT, C5; CHLOROFOM EXTRACT, D5; ETHYL ACETATE EXTRACT, E5; METHANOLIC EXTRACTS]

FIG 36

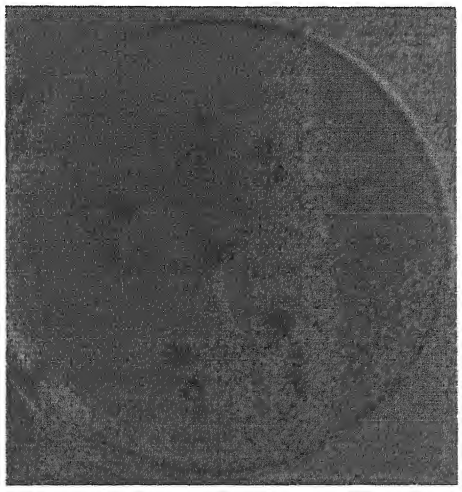
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [2 mg/ml] AGAINST S. AURIUS



[A2; PETROLEUM ETHER EXTRACT, B2; BENZENE EXTRACT, C2; CHLOROFOM EXTRACT, D2; ETHYL ACETATE EXTRACT, E2; METHANOLIC EXTRACTS]

FIG 37

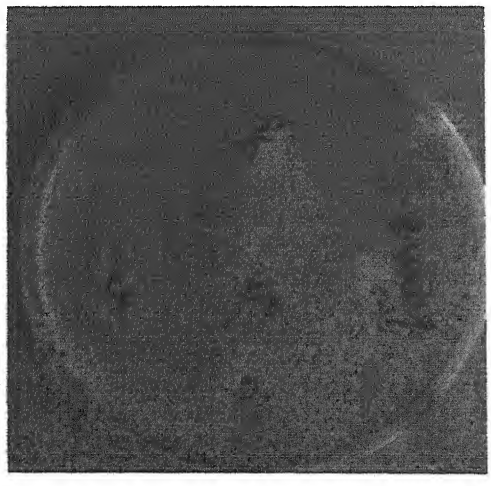
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [8 mg/ml] AGAINST S. AURIUS



[A4; PETROLEUM ETHER EXTRACT, B4; BENZENE EXTRACT, C4; CHLOROFOM EXTRACT, D4; ETHYL ACETATE EXTRACT, E4; METHANOLIC EXTRACTS]

FIG 38

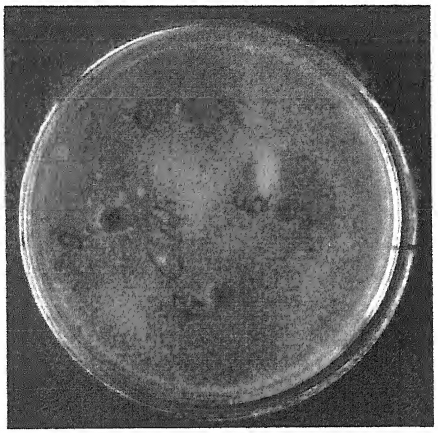
ANTIBACTERIAL ACTIVITY OF CARISSA CARANDAS ROOT EXTRACTS [32 mg/ml] AGAINST S. AURIUS



[A6; PETROLEUM ETHER EXTRACT, B6; BENZENE EXTRACT, C6; CHLOROFOM EXTRACT, D6; ETHYL ACETATE EXTRACT, E6; METHANOLIC EXTRACTS]

FIG 39

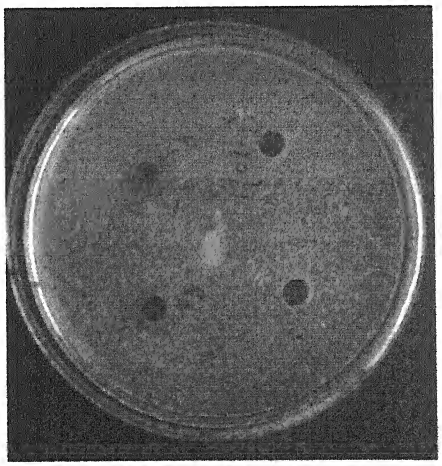
DETERMINATION OF MINIMUM INHIBITION CONCENTRATION OF BENZENE EXTRACT AGAINST E. COLI



[F6; 0.025 mg/ml; F2 0.05 mg/ml; S standard; C Control]

FIG 40

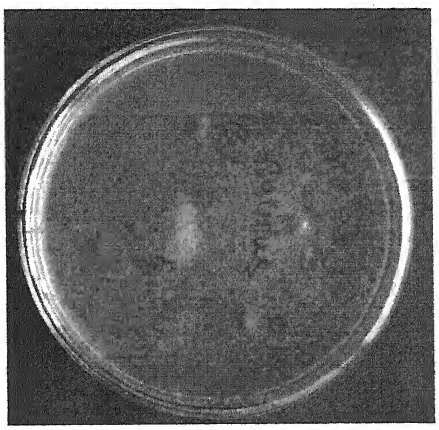
DETERMINATION OF MINIMUM INHIBITION CONCENTRATION OF BENZENE EXTRACT AGAINST E. COLI



[F3; 0.1 mg/ml; F4 0.2 mg/ml; S standard; C Control]

FIG 41

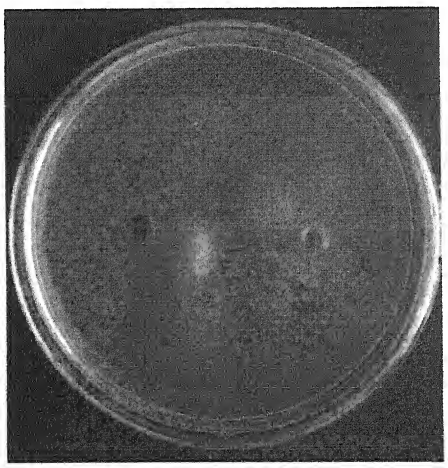
DETERMINATION OF MINIMUM INHIBITION CONCENTRATION OF BENZENE EXTRACT AGAINST B. SUBTILIS



[F6; 0.025 mg/ml; F2 0.05 mg/ml; S standard; C Control]

FIG 42

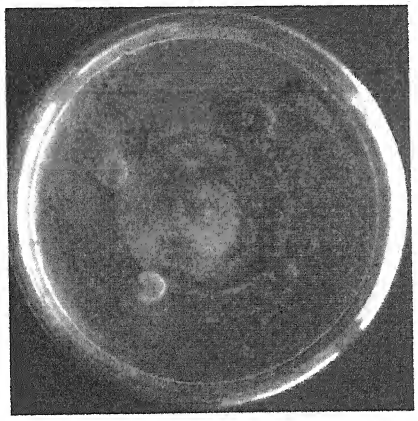
DETERMINATION OF MINIMUM INHIBITION CONCENTRATION OF BENZENE EXTRACT AGAINST B. SUBTILIS



[F3; 0.1 mg/ml; F4 0.2 mg/ml; S standard; C Control]

FIG 41

DETERMINATION OF MINIMUM INHIBITION CONCENTRATION OF BENZENE EXTRACT AGAINST S. AURIUS



[F6; 0.025 mg/ml; F2 0.05 mg/ml; S standard; C Control]

FIG 42

ANTIFUNGAL ACTIVITY OF CARISSA CARANDAS ROOT METHANOLIC EXTRACT [16 mg/ml] AGAINST *A. FUMIGATUS*

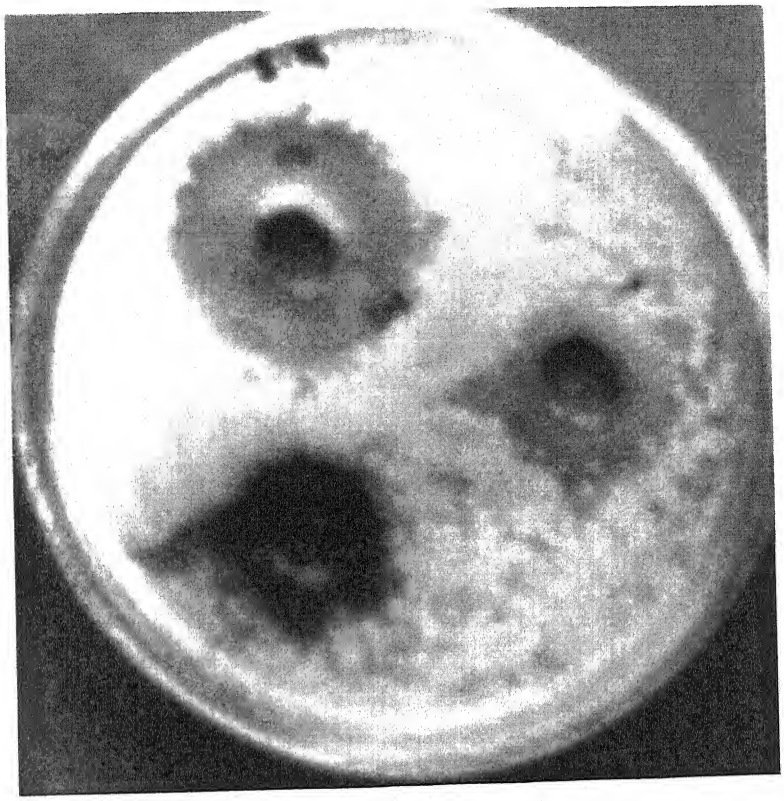
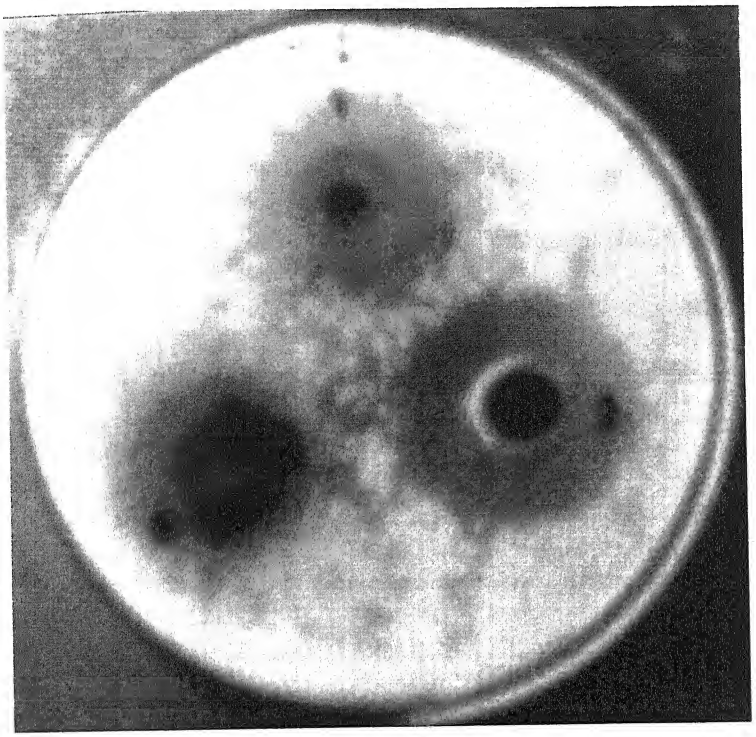


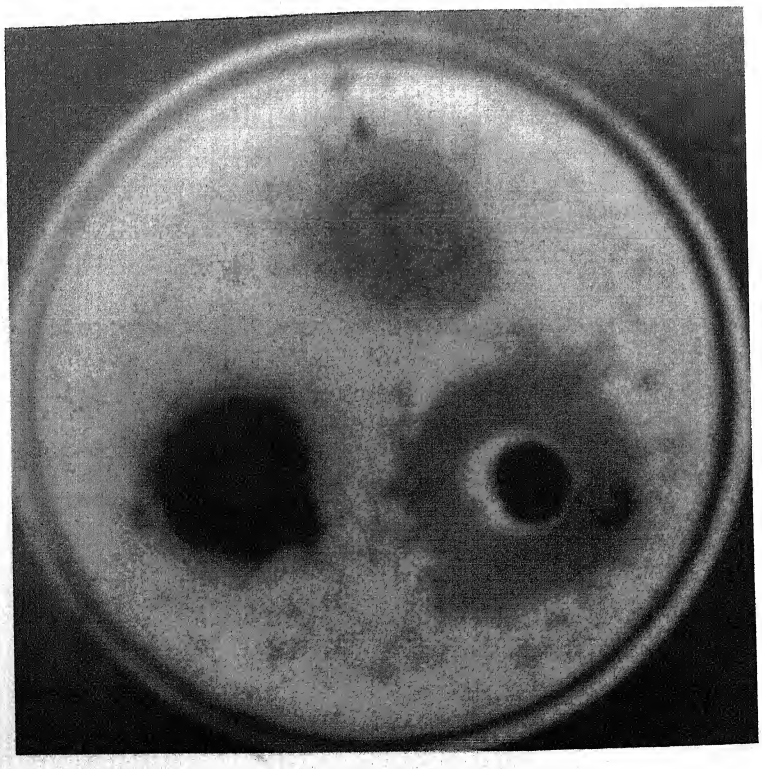
FIG 43

ANTIFUNGAL ACTIVITY OF CARISSA CARANDAS ROOT METHANOLIC EXTRACT [32 mg/ml] AGAINST *A. FUMIGATUS*



G 44

ANTIFUNGAL ACTIVITY OF CARISSA CARANDAS ROOT METHANOLIC EXTRACT [16 mg/ml] AGAINST *A. NIGER*



ANTI-STRESS AND ADAPTOGENIC ACTIVITY

Stress represents a reaction of the body to a stimulus that tends to alter its normal physiological equilibrium or homeostasis and has been defined as a nonspecific response of the body to any demand imposed on it (Sreemantulal, et al., 2005) and elicits various endocrinal and visceral changes in plasma cortisone and gastric mucosal integrity (Bhatwadekar et al., 1999). To successfully combat stress and stressful situations, adaptation is required. Adaptation might be best thought of as the ability to be exposed to a stressor, while responding with either decreased no characteristic hormonal perturbations. Adaptation also implies being prepared to and capable of rapidly reassuming homeostasis after the stressor is withdrawn (Gregory and Rhodiolarosea 2001). An adaptogen produces a non-specific response in an organism; i.e, an increase in power of resistance against multiple stressor including physical, chemical, or biological agents. An adaptogen also has normalizing influence on physiology, irrespective of the direction of change from physiological norms caused by the stressor. However it is incapable of influencing normal body functions more than required to gain non-specific resistance. (Brekhman and Dardymov, 1969) since the introduction of adaptogen, several plants have been investigated, which were once used as tonics due to their adaptogenic and rejuvenating properties in traditional medicine. The drugs of plant origin are gaining increasing popularity and are being investigated for remedies of a number of disorders including anti-stress activity. Plant adaptogens cause our physiology to begin the adaptation process to stress. When a stressful situation occurs, consuming adaptogens generates a degree of generalized adaptation that allows our physiology to handle the stressful situation in a more resourceful manner.

1. The *Carissa carandas L* was being favored by British residents in India as reminiscent of gooseberries (The wealth of India, 1992; Kartikar and Basu,1984; Nadkarni,1954).

The purpose of the present study is to evaluate adaptogenic and anti-stress activity of *Carissa carandas* root in rats.

Animals:

All animals being used in the study were breed and raised, under the facility of animal house of the H.S.K. college of Pharmacy at Bagalkot. Female Wistar albino rats of sex weighing 150-180 g were used for anti-diarrheal and anti-ulcer study and male swiss albino mice weighing 15-18 g were used for gall bladder emptying study. All animals were fed standard pellet and tap water *ad libitum* before the experiments. All the experiments were conducted in accordance with the Institutional Animals Ethics Committee after the approval.

Antistress activity:

Group of six female wistar rats per dose of test drug and for controls weighing 200-250 gm were used . The extract was administered continuously for 14 days time and animals were restrained in polypropylene rats restrainer in prone position for a period of one hour daily lasting for 15 days. The Anti-stress activity was evaluated by using ulcer score and ulcer index and treated groups were compared with control. Finally animals were sacrificed, stomach was removed, fixed on cork plate and the number and severity of ulcer were registered with a lens using the following score o=no ulcer; 1= superficial ulcer; 2= deep ulcer; 3= perforation. The ulcer index was calculated by the formula, $U_I = Us + U_N + U_P \times 10^{-1}$ where U_N = average number of ulcer per animal, Us = average of severity score and U_P =percentage of animals with ulcers (Nagraj and Jaganathan. 1999).

Adaptogenic activity:

Adaptogenic activity was evaluated by following Sharma, et al 1989 method with little modification; briefly, the animals were grouped into four, six animals in each group, one is control and other three groups were treated, control grouped received propylene glycol (1% body weight, PO.) and other treated groups received extract of 100, 200, and 500 mg/kg body weight (P.O.) respectively. The drug was administered 1 hour earlier to animals forced for swimming. Wister albino rats of body weight 200 to 250 gm were individually subjected to swim in vertical Plexiglas's cylinder (height: 40 cm diameter 18 cm, containing 15 cm of water). Duration of swimming was recorded for a period of 14 days and compared with control group.

Statistical Evaluation:

The results are expressed as mean ± standard error of mean (S.E .M). The results obtained from this study were evaluated by students' t test doses were compared with control group and ulcer index was calculated along with scoring the severity of ulcer.

RESULTS

Adaptogenic activity:

Table 10 shows that, the administration of maximum dose of extract produced a significant ($P<0.01$) increase in the swimming duration as compared to the control from day to till the completion of study. A dose of 200 mg /kg did not produce significant change in duration of swimming, and mean duration of swimming time was without significant difference than compared to control group. The small dose of the extract 100 mg /kg gradually improved the performance and significantly increased the duration of swimming on 14 days of study than compare to control.

Antistress activity:

Table 11 shows that extract pretreatment on restrained rats produced a significant reduction in severity of ulcer as well as ulcer index. All the doses selected in the study significantly ($p < 0.5$ and $P < 0.01$) reduce the severity of ulcer by reducing the ulcer score. However, 100 mg /kg dose of the extract decreased the ulcer index but moderate and high dose of extract failed to decrease the ulcer index than compared to control. The 200 mg/kg and 500-mg/kg dose of the extract also failed to overcome the hemorrhage and to reduce the total mucosal area affected in restrained stress.

DISCUSSION

Stress is the one of the silent killing disorder, which is also, associated with several neurochemical, psycobehavioural, physiological, pathological, and pathophysiological and biochemical changes (Dobrakovova et al.1990). Stress, age and behavior characteristics are considered to be risk factors for disturbances of the cardiovascular system in animal and man. It has been suggested that chronic stress can contribute to the development of exacerbation of cardiovascular dysfunction (Pitman et al. 1988). Exposure to various stressful stimuli triggers the activation of hypothalamus pituitary adrenal (HPA) axis and the sympathetic adrenomedullary system. There is considerable evidence showing that HPA response is progressively reduced after repeated exposer to the same stressor. This phenomenon called adaptation was shown to occur following exposure to various chronic intermittent stressors as physical restraint (Pitman et al. 1988, Sely. 1976). Stress has been suspected to be one of the mechanisms leading to disease under certain circumstances (Nagraj and Jaganathan. 1999), several studies demonstrated that certain plant extracts and some of the glycosides obtained from these produce a state of non- specifically increased resistance in animals and human beings (Lobo and Desai.1975). Adaptogens are known to induce cellular regeneration, prevent arteriosclerosis, increase hormone

utilization enhance protein and carbohydrate metabolism and produce reduction in serum lipid (Sahgal and. Geriforte. 1975). Besides it has been proved useful clinically by producing a feeling of well-being, increasing mental activity, lessening fatigue, increasing appetite and sexual function in the ageing (Bhattacharya and Bhattacharya. 2000, Singh et al. 2001, Singh et al 1978) The stimulant action of coca leaves was explained as due to the presence of the alkaloid cocaine or and their metabolites.

All the parts of the plant are reputed in indigenous medicine. The roots credited with bitter, stomachic and anthelmintic properties. The alcoholic extract of roots exhibited cardiotonic activity and deep and prolonged blood pressure lowering effect. The roots contain carissone, carindone, carinol, and sitosterol. An amorphous water –soluble polyglycoside fraction possessing significant cardiac activity has also been isolated; the hydrolysis of the fraction gave odoroside H, digitoxigenin glucose and D- digitalose. The cardiotonic activity of water-soluble fraction has been attributed to the presence of glucosides of odoroside H. Presence of alkaloids is also reported in root and stem bark (The wealth of India.1992).

In the present study we evaluated the adaptogenic property of petroleum ether extract of *Carissa carandas* root. We used the swimming endurance model for preliminary evaluation of its possible adaptogenic property and the period of swimming was considered as the parameter .The small dose and maximum dose of the extract selected for study has shown dramatic effects than compare to higher doses. The small dose progressively improved the performance till the 14 days of study, however higher dose significantly increased the performance from day 1 of the study and continued till the last day of study. The increased performance of the extract indicates its possible presence of adptogenic properties in it. Therefore, a rational approach was made to evaluate its antistress properties. For this, restraint stressful

situation was induced in animals. Improved swimming duration and prevention of stress- induced in ulcers indicate the anti stress properties of *Carissa carandas*. These activities could be mediated due to the presence of flavones, terpenoids, alkaloids, and glycosides.

We conclude from the present study that, low dose of the extract is safe and may be used safely for the improvement of performance because of presence of anti stress properties. In this study the extract reduced totals ulcer scores significantly and dose dependently but the moderate and maximum dose failed to reduce ulcer index significantly than compare to control. The herbal extract at minimum dose may be safe and beneficial in coping up the stressful condition and to improve and maintain vitality, increased efficiency immunity of the body.

TABLE-9
ACUTE TOXICITY OF *CARISSA CARANDAS* ROOT METHANOLIC EXTRACTS ON SWISS ALBINO MICE

Drug	Dose	Toxicity		Time of Death (hrs)	Observations										
		Onset (hr)	Stop (hr)		Skin & fur	Eyes	Respiration	CNS	Tremors	Convuls ions	Salivation	Diarrhea	Sleep	Lethargy	Coma
Methanolic Extract	3mg/kg	—	—	—	X	X	X	††	X	X	X	X	X	X	X
	30mg/kg	—	—	—	X	X	X	X	X	X	X	X	X	X	X
	300mg/kg	---	---	-----	X	X	X	††	X	X	X	X	^^	X	X

Duration of study: 15 days

Negative, †† = CNS stimulation

TABLE 10

ADAPTOGENIC ACTIVITY OF *CARISSA CARANDAS* ROOT USING SWIMMING MODEL

TRETMENT	1 DAY	2 DAY	4 DAY	7 DAY	14 DAY
CONTROL (1% BW Propylene glycol)	1.1 ± 0.14	1.30 ± 0.07	1.39 ± 0.15	1.14 ± 0.1	1.01 ± 0.18
Extract 100mg/kg	0.31 ± 0.06	0.5 ± 0.074	1.63+-0.17	1.18 ± 0.048	2.70 ± 0.4 **
Exract 200mg / Kg	0.85 ± 0.08	0.86 ± 0.03	1.14 ± 01.5	1.08 ± 0.16	1.7 ± 0.37
Extract 500mg /kg	2.18 ± 0.33 *	1.58 ± 0.47	2.1 ± 0.36 **	2.89 ± 0.48**	3.1 ± 0.6 **

The extract was administered 1 hrs prior to forcing the rats for swimming. Results are presented as Mean ± SEM and analyzed by students T test . * P<0.05 , **P<0.01.

TABLE 11

ANTI-STRESS OF *CARISSA CARANDAS* ROOT USING RESTRAIN STRESS MODEL

Treatment	Ulcer index	Ulcer score
Control	1.08 $\pm$ 0.01	3.3 $\pm$ 0.3
Extract 100 mg	0.86 $\pm$ 0.07**	1.7 $\pm$ 0.6**
Extract 200mg	1.07 $\pm$ 0.07	2.3 $\pm$ 0.6 *
Extract 500mg	1.07 $\pm$ 0.06	2.4 $\pm$ 0.8*

The extract was administered 1 hr prior to restraining the rats . Results are presented as Mean $\pm$ SEM and analysed by student t test . * $P<0.05$, **$P<0.01$.

ANTI-DEPRESSANT AND ANTI-ULCER ACTIVITY

Depression is a heterogeneous disorder that has been characterized and classified in a variety of ways. Major depression is one of the most common psychiatric disorders. At any given movement, about 5-6% of the population is depressed and an estimated 10% of people may become depressed during their lives (Cooper, et al 2003). Depression and other serious affective manifestations may account for as many as 50% of those hospitalized for psychiatric reasons. Bipolar depression to extreme relation, occurs slightly more often in women then men, shows strong familial patterns of inheritability, and is primarily found in young adults aged 18-45. Unipolar and major depression is characterized by one or more prolonged episodes of depression, occurs for more frequently in the population than the bipolar form, and is nearly three times as common in women as in men1. Despite intensive research, the pathogenesis and mechanism of action of various pharmacological agents are still not understood. None of the newer antidepressant has been shown to be more effective over all than the tricyclics with which they have been compared. However, tricyclics have failed to produce speedy action and requires long term treatment. Most of the newer and old synthetic drugs like TCA produce its action by increasing biogenic amines (Adr. NA, DA, 5-HT) at the receptor sites but these drugs enjoying major side effects like anticholinergic action, sedation, mental confusion, weight gain, hypomania, mania, sweating and fine tremors, lower seizure thresholds, cardiac arrhythmias etc (Poller et al., 2004). Hence, the present study was conceived.

The objective of the present study was to evaluate antidepressant property of *Carissa carandas* root extract with gastroprotective effects. C. carandas Linn is a large evergreen shrub with a short atom, glabrous, expect the inflorescence; bark light gray, scaly; branchlets usually alternate, with thin sharp horizontal glabrous spines 2.5-3.8

cm. long at there bases3. The root is anthelmintic, the fruit is sour, acrid; astrigent, appetizer; lessons thirst, biliousness; useful in the disease of brain (Kartikar et al., 1984). The root has reputation of bring a better stomachic. Used in the konkan, pounded with hoarse urine, lime juice and camphor as remedy for itch. In Cuttack, the decocation of the leaves is very much used at the commencement of the remittent fever (Kartikar et al., 1984; Nadkarni, 1954; The wealth of Indian 1992).

All parts of the plant are reputed in indigenous medicine. The roots are credited with bitter, stomachic and anthelmintic properties. The alcoholic extract of roots exhibited cardiotonic activity and a deep and prolonged blood pressure lowering effect. The roots contain carissone, carindone, carinol, sitosterol. An amorphous water-soluble polyglycoside fraction possessing significant cardiac activity has also been isolated; the hydrolysis of the fraction gave odoroside H, digitoxigenin, glucose and D-digitalose. The cardiotonic activity of water soluble fraction has been attributed to the presence of glucosides of odorosie H. Presence of alkaloids is also reported in root and stembark (The wealth of Indian 1992).

MATERIAL AND METHODS

Plant Preparation:

The root of *Carissa carandas* was collected, dried and cut into pieces, crushed into powder then passed through sieve no 40 to obtain uniform particle and extracted successively with the solvents of increasing polarity Petroleum Ether, Benzene, Chloroform, Ethyl acetate, Methanol, by successive solvent extraction method. For each solvent 12 hours cycle of extraction was carried out and concentrated by flash evaporator, dried at low temperature in freeze dryer under vacuum. The extract was reconstituted in propylene glycol as a solvent (Jain et al 2005).

Animals:

All animals being used in the study were breed and raised, under the facility of animal house of the H.S.K. college of pharmacy at Bagalkot. Wister albino rats of either sex weighing 150-180 g were used for antidepressant and antiulcer activity. All animals were fed standard pellet and tap water ad libitum before the experiments. All the experiments were conducted in accordance with the Institutional Animals Ethics Committee after the approval.

Methods:

Antidepressant activity:

Despair swim test was used with little modification as animal model for evaluation of antidepressant activity, in brief rats were forced to swim in a restricted space from which they cannot escape are induced to characteristic behavior of immobility. This behavior reflects a state of despair which was reduced by several agents which are therapeutically effective in human depression.

Male wistar rats weighing 160–180 g were used. They were brought to the laboratory 24 h before the experiment and were housed separately in standard cages with free access to food and water. Naive rats were individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25°). After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water the rats were removed and allowed to dry in a heated enclosure (32°) before being returned to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility was measured during a 5 min test. Floating behavior during this 5 min period was found to be reproducible in

different groups of rats. An animal was judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. The study was continued for fourteen days and the treatment with extract was started from day one till the last day of observation. Total duration of immobility was calculated and compared with propylene glycol treated group (Wallach and Hadley 1979).

Antiulcer activity:

Pylorus legation method with little modification was used as animal model for evaluation of antiulcer activity. Female wistar rats weighing 150–170 g were starved for 48 h access to drinking water ad libitum. During this time they were housed single in cages in order to avoid cannibalism and coprophagy. Six animals were used per dose and as controls. Under ether anesthesia a midline abdominal incision was made. The pylorus was ligated, care had been taken that neither damage to the blood supply nor traction on the pylorus occurs, the abdominal wall was closed by sutures. The extract and solvent were given orally by gavages and the animals were placed for 19 h in rat restrainers. Afterwards, the animals were sacrificed in ether anesthesia. The abdomen was opened and a ligature was placed around the esophagus close to the diaphragm. The stomach was removed, and the contents were drained in a centrifuge tube. Along the greater curvature, the stomach was opened and pinned on a cork plate. The mucosa was examined with a dissection microscope. The lesions were occurred mainly in the rumen and in the atrium in the control animals. The number of ulcers was noted and the severity was recorded with ulcer scores and ulcer index (Shay et al., 1954).

The volume of the gastric content was measured. After centrifugation, acidity was determined by titration with 0.1 N NaOH. Ulcer index, gastric secretion and acidity of the gastric content of treated animals were compared with controls.

RESULTS

Antidepressant Activity:

Results obtained in this study are represented in Table 12. The maximum dose of the extract has shown promising and significant ($P<0.05$) decrease in duration of immobility phase when compared to control from sixth day to the last day of study. However, the minimum and moderate doses selected for the present study have failed to significantly decrease the immobility phase in despair swim test (Table 12).

Antiulcer Activity:

The gastric secretion was significantly ($p < 0.05$) inhibited at low dose and moderate dose of the extract and present inhibition was 71.79 and 78.63 respectively, however, maximum dose 500 mg/kg was failed to inhibit the gastric secretion significantly than compare to control. The 125-mg/kg dose of the extract reduced the free acidity higher than the free acidity reduced by ranitidine. Extract at 125 mg/kg and 250 mg/kg reduced the ulcer index significantly than compare to control. The ulcer score obtained in the present study indicates that, the 125-mg/kg dose of the extract significantly reduced the ulcer score than compare to moderate and maximum dose of the extract (Table 13).

DISCUSSION AND CONCLUSION

The exact etiology of depression is unknown numerous factors appear to contribute. These include genetics, life/event sensitization and biochemical changes. In addition, stressful life events can contribute to depression; most studies concur that the likelihood of a depressive episode is five to six times greater six months after events

such as early parental loss, job loss or divorce. The link between depression and stressful life events has been conceptualized in the form of the sensitization model, which proposes that prior exposure to stressful life events sensitizes the brain's limbic system to the degree that subsequently less stress is needed to produce a mood disorder (Freeman et al., 1993). Many of the current biochemical theories of depression focus on the biogenic amines, which are a group of chemical compounds important in neurotransmission. Antidepressant medication, which address the brain's biochemistry, include MAO inhibitors, tricyclic antidepressants and selective serotonin reuptake inhibitors. MAOs increase norepinephrine levels, while tricyclics essentially enhance norepinephrine transmission (Rush et al., 1991).

The current therapeutic goal in the treatment of major depression is to improve the quality of life by normalizing mood, increasing awareness of personal pleasures and interests and reversing the functional and social disabilities associated with depression, as well as to reduce suicide rates (Judd, 1995). *C. Carandas* is used in folk medicine as an astringent, antiscorbatic, remedy for biliousness, in cases of intermittent fever, diarrhea, oral inflammation and earache. The root is employed as a bitter stomachic and vermifuge and it is an ingredient in a remedy for itches. The roots contain salicylic acid and cardiac glycosides causing a slight decrease in blood pressure. Also reported are carissone; the D-glycoside of B-sitosterol; glucosides of odoroside H; carindone, a terpenoid; lupeol; ursolic acid and its methyl ester; also carinol, a phenolic lignan. Bark, leaves and fruit contain an unnamed alkaloid (Kartikar et al., 1984; Nadkarni, 1954; The wealth of Indian 1992). British residents in India undoubtedly favored the karanda as being reminiscent of gooseberries (Nadkarni, 1954). The present study was carried out to investigate the possible antidepressant effect of *C. carandas* compared with placebo in the treatment of major

depression. Rats were placed in the cylinders for 15 min, the first time the rats were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom and total immobility period was noted. Extract was administered one hour prior to testing. Since experiments with the administration of extract showed that the test gave the most stable results in reducing immobility these times are chosen for the experiment (Wallach and Hedley, 1979). Antiulcer activity was also considered in this study to find out possibility to use this drug in the treatment of stress associated depressive conditions.

Several plants and their constituents including St. John's wort (De Smet, 1996), Piper methysticum (Singh, 1992), Ginkgo biloba (Kleijnen J and Knipschild, 1992), Saffron (Rios et al., 1996), Crocus sativus (Karimi et al., 2001), etc have been studied in detail. They had been found to produce antidepressant activity through inhibition of MAO A and B, neuronal uptake of several neurotransmitters by increasing Na+/H+ exchange and increased intracellular Na+ level due to the presence of important phytochemicals like flavonglycosides and (De Smet, 1996; Singh, 1992; Kleijnen J and Knipschild, 1992; Rios et al., 1996; Karimi et al., 2001). These plants shown to produce improvements in many psychological symptoms, including depression, anxiety, apathy, sleep disturbances, insomnia, anorexia and feelings of worthlessness (De Smet, 1996; Singh, 1992; Kleijnen J and Knipschild, 1992; Rios et al., 1996; Karimi et al., 2001).

In this small preliminary evaluation the maximum dose was found to be effective. Our results are in the line with earlier animal studies of herbal crude extracts and our study (Patil et al., 2006). However, the higher dose failed to produce gastroprotective property, and indicating its rare chances to use in the treatment of stress associated depressive conditions. The earlier studies indicate that the active constituents include

the terpenoids, flavonoids, glycosides and flavonglycosides and may be responsible for antidepressive and antiulcer activities. Probably the actions could be due to unspecific inhibitors of the neuronal uptake of several neurotransmitters, increasing synaptic level of the biogenic amines and inhibition of monoamine oxidase.

In majority of cases, the failure of conventional medicines was observed and leading to consider the non-conventional and alternative medicines in the treatment of depression. Therefore, the search for new and more effective therapeutic agents includes the scientific study of plants used in traditional medicine systems to treat mental disorders. The main overall finding from this study is that *C. carandas* may be of therapeutic benefit in the treatment of mild to moderate depression not associated with stress. Further, large-scale trial study with various doses and models is required to establish its possibility of application

TABLE-12

ANTIDEPRESSANT ACTIVITY OF CARISSA CARANDAS LINN USING DESPAIR SWIM TEST

Days of observation (Duration of Immobility Phases in Seconds)						
Treatment	1 Day	2 Day	4 Day	6 Day	12 Day	14 Day
Propylene glycol	86 ± 6.7	90 ± 5.75	101 ± 10.45	97 ± 6.86	102 ± 2.22	105 ± 0.17
Extract (125 mg/kg)	88.33 ± 18.93	88.2 ± 17.15	71.66 ± 9.84	74.5 ± 7.5	79.5 ± 6.77*	80 ± 19.78
Extract (250 mg/kg)	71.25 ± 11.26	98 ± 8.51	111.33 ± 14.83	97 ± 6.98	87.5 ± 13.23	74.83 ± 8.78
Extract (500 mg/kg)	82.5 ± 8.85	50.5 ± 10.29	73.83 ± 7.96	66 ± 1.02*	65.83 ± 0.93**	62.82 ± 3.1**

The results are expressed as Mean ± standard error of the mean. The results were analysed by Student 't' test and compared with control (Propylene glycol treated) for coming to conclusion. $P<0.05$ was considered as significant. *$P<0.05$, **$P<0.001$

TABLE 13

ANTIULCER ACTIVITY OF CARISSA CARANDAS LINN USING PYLORIC LIGATION TEST

Treatment	Gastric Secretion	% Inhibition of Gastric Secretion	Free Acidity	Ulcer Index	Ulcer Score
Propylene glycol 2 ml/kg	2.34 ± 0.74	--------	0.072 ± 0.008	0.9 ± 0.2	3.3 ± 0.3
Ranitidine	0.98 ± 0.3	58.11	0.05 ± 0.01	-------	------
Extract (125 mg/kg)	0.66 ± 0.13*	71.79	0.045 ± 0.2	0.16 ± 0.08*	1.7± 0.6
Extract (250 mg/kg)	0.5 ± 0.03*	78.63	0.058 ± 0.02	0.13 ± 0.03*	2.3 ± 0.6
Extract (500 mg/kg)	1.45 ± 0.13	38.03	0.06 ± 0.01	0.44 ± 0.11	2.4 ± 0.8

The results are expressed as Mean ± standard error of the mean. The results were analysed by Student 't' test and compared with control (Propylene glycol treated) for coming to conclusion. $P<0.05$ was considered as significant. *$P<0.05$

ANTI-DIARHOEAL ACTIVITY

Diarrhea accounts for more than 5-8 million deaths each year in infants and small children less than 5 year. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhea and it has been recognized as one of the most important health problems in developing nations (Snyder and Merson, 1982; Park, 2000; Fontaine, 1988; Aranda-Michel et al., 1999).

The *C. carandas L.* commonly known as karanda is native and common throughout much of India, Burma, and Malacca and area of Ceylon; is rather commonly cultivated in these areas as a hedge and for its fruits and the fruit is marketed in villages. In Asia, the ripe fruits are utilized in curries, tarts, puddings and chutney. When only slightly under ripe, they are made into jelly. Green, sour fruits are made into pickles in India. With skin and seeds removed and seasoned with sugar and cloves, they have been popular as a substitute for apple in tarts. British residents in India undoubtedly favored the karanda as being reminiscent of gooseberries (Kartikar et al., 1984; The Wealth of India, 1992; Nadkarni, 1954).

The unripe fruits are used medicinally as an astringent. The ripe fruits is taken as an antiscorbatic and remedy for biliousness. The leaf decoction is valued in cases of intermittent fever, diarrhea, oral inflammation and earache. The root is employed as a bitter stomachic and vermifuge and it is an ingredient in a remedy for itches. The roots contain salicylic acid and cardiac glycosides causing a slight decrease in blood pressure. Also reported are carissone; the D-glycoside of B-sitosterol; glucosides of odoroside H; carindone, a terpenoid; lupeol; ursolic acid and its methyl ester; also carinol, a phenolic lignan. Bark, leaves and fruit contain an unnamed alkaloid (Kartikar et al., 1984; The Wealth of India, 1992; Nadkarni, 1954).

Castor oil induced diarrhea:

Rats were divided into eight groups (n = 6) and, fasted for 18 h and water was provided *ad libitum*. The methanolic extract of *Carrissa Carandas* (50, 100 and 200 mg/kg) were administered orally to the first three groups of rats. One group received 10 ml/kg noramal saline and served as a negative control. Another group received the standard drug Atropine (5 mg/kg, p.o.) as positive control. After 1 h of treatment, all the animals were challenged with 1 ml of castor oil orally, by gavage and observed for consistency of faecal material (Venkateshan, et al., 2005). The frequency of defecation was noted in transparent plastic dishes placed beneath the individual rat cages upto 6 h 9.

Intestinal transit:

Rats were divided into four groups (n = 4) and fasted for 18 h before the experiment. Each animal was orally administered with methanolic extracts to the first three groups of animals in the dose of 50, 100 and 200 mg/kg. The fourth group was treated with normal saline (10 ml/kg, p.o.) and served as a negative control. The fifth group received atropine (5 mg/kg, i.p.), as the positive control. Thirty minutes later, each animal was orally administered with 1 ml of charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth), after thirty minutes of oral administration of charcoal meal, animals were killed and the intestinal distance moved by the charcoal meal from the pylorus to caecum was measured and expressed as percentage of distance moved (Chitme et al., 2004).

Gall bladder emptying:

Male Swiss albino mice were divided into four groups of ten each and were starved for 24 h before the experiment with free access to water (Basanagouda, 2004).

Biliary motility was determined by the method of Valsecchi & Toson G (1982). Animals of one group received normal saline by intraperitoneal (5 ml/kg) and *C. Carandas* methanolic extract oral (50, 100 and 200 mg/kg) routes to establish the effect of extract on normal weight of the gall bladder. Gall bladder emptying was induced by oral administration of 1ml of a 30% suspension of lyophilised egg yolk in saline, 8 min after intraperitoneal administration of drug or saline. Animals were killed by ether inhalation 15 min after oral administration of egg yolk. Gall bladders were removed by sectioning the cystic duct and were weighed. The standard weight of the gall bladder was calculated for each group. Percent inhibition of the emptying was calculated as:

% Inhibition of emptying = (Ti-C) x100/B-C

Where, B = mean weight (mg) of gall bladder in control, C = mean weight (mg) of gall bladder in the egg-yolk-treated control group, Ti = mean weight of gall bladder (mg) in the drug-treated group.

Statistical Evaluation:

The results are expressed as mean ± standard error of mean (S.E.M.). The results obtained from this study were evaluated by students 't' test. The test doses were compared with control group and ulcer index was calculated along with scoring the severity of ulcer. A value of $P<0.05$ was considered statistically significant.

RESULTS

Gall bladder emptying:

The mean weight of the gall bladder in saline-treated mice was 32.22±0.611 mg. Oral administration of egg yolk resulted in emptying of the gall bladder and the average weight of the gall bladder was 7.67±0.829 mg. Oral administration of methanolic extract of *C. carandasa* (50, 100 and 200 mg/kg) inhibited the egg yolk-induced gall bladder emptying in a dose-dependent manner; there was a significant (*P<0.001*) increase in the average weight of the gall bladder of these groups when compared with control (Table 14).

Castor oil induced diarrhea:

Total fecal output studied for six hours has shown that the extract treated group significantly decreased the fecal output than compare to control (P<0.01). The extract of *C. carandas* has also decreased the intestinal transit than compare to control (P<0.01). The effect of the extract was much higher than the standard (Table 15).

Charcoal meal test

Oral administration of 1 ml of castor oil induced increased intestinal transit of charcoal meal 91.87 ± 1.975 cm. Atropine 44.1 percent inhibited the castor oil induced increased intestinal transit. Methanolic extract dose dependently decreased the intestinal transit at mild and moderate dose 60.5 and 72.95 respectively, whereas high dose of extract failed to inhibit the transit and was 17.86 percent then compared to control saline treated group (Table 16).

DISCUSSION:

Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhea and dysentery, of the indigenous plants used, *Andrographis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata*, and others are mentioned (Singh, 1992). Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract. In the present study, methanolic extracts of *Carrissa carandas* that have not been studied so far, was evaluated for its anti-diarrheal potential against castor oil induced diarrhea and gastrointestinal motility in charcoal meal test in female albino Wistar rats.

The methanolic extract of *Carrissa carandas* exhibited significant anti-diarrheal activity against castor oil induced diarrhea in rats. The extracts are in crude form but had a significant activity than atropine, when tested at 200 mg/kg, statistically significant reduction in the frequency of defecation and the wetness of the faecal droppings was observed when compared to untreated control rats. It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhea (Ammon et al., 1974; Gaginella et al., 1975).

In our unpublished preliminary study, both extracts exhibited significant anti-inflammatory activity in the carrageenan-induced rat paw oedema. Based on these observations, it seems reasonable to suggest that the anti-diarrheal effect of methanolic extracts may be due to the inhibition of prostaglandin biosynthesis.The extract appears to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at 50 and 100 mg/kg,

similar to that of atropine. Previous study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption (Levy, 1982). Thus, gastrointestinal motility test with activated charcoal was carried out to find out the effect of methanolic extracts on peristaltic movement. The results also show that the methanolic extracts suppressed the propulsion of charcoal meal thereby increased the absorption of water and electrolytes. The inhibitory effect was also supported by gall bladder emptying study.

The presence of egg yolk in the duodenum releases CCK, which is a physiological stimulus for gall bladder emptying by inducing contraction of the gall bladder muscle and relaxing the Sphincter of Oddi (Malave and Yim 1992; Staritiz, 1988; Byrnes et al., 1981). In the present study pre-treatment of mice with saline has no effect on egg-yolk-induced gall bladder emptying. Methanolic extract of Carissa carandasa in a dose-dependent manner inhibited egg yolk induced gall bladder emptying suggesting the applicability of the plant preparation in the treatment of the undesirable gastrointestinal effects associated with therapeutic administration or with the compulsive use of narcotic analgesics (Reisine and Pasternak, 1996).

Previous reports have demonstrated the anti-diarrheal activity of tannin, flavonoids, alkaloids, saponins, reducing sugars and sterols and/or terpenes, containing plant extracts (Kleijnen and Knipschild, 1992; Rios et al., 1996; Reisine and Pasternak, 1996)

The phytochemical analysis of the extracts showed the presence of alkaloids, saponins, flavonoids, sterols and terpenes and sugars. These constituents may responsible for the anti-diarrheal activity of *Carrissa carandas* extracts.

The results indicate that the methanolic extract of *Carrissa carandas* possesses significant anti-diarrheal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. The inhibitory effect of the extract justified the use of the plant as a non-specific anti-diarrheal agent in folk medicine. Further detailed investigations are underway to determine the exact phytoconstituents which are responsible for the anti-diarrheal activity.

In this small preliminary evaluation the maximum dose was found to be effective. Our results are in the line with earlier animal studies of herbal crude extracts. The main overall finding from this study is that *C. carandas* may be of therapeutic benefit in the treatment of diarrhea and gastroprotection (at small dose). Further, large scale trial study with various doses and models is required to establish its possibility of application

TABLE 14

EFFECT OF *CARISSA CARANDAS* METHANOLIC EXTRACT ON GALL BLADDER EMPTYING

Animal No	Treatment				
	Control	Control Saline i. P.	ME 50 mg/kg	ME 100 mg/kg	ME 200 mg/kg
1.	30.2	8.2	17.6	14.1	14.2
1.	31.5	8.65	16.2	11.6	10.6
2.	33	6.98	18.2	11.8	9.8
3.	34.2	7.54	19.1	15.6	10.2
4.	33.01	6.48	15.2	15.8	12.1
5.	31.	8.21	17.2	13.7	12.6
Mean	32.2	7.67	17.3	13.8	11.6
SEM	0.611	0.829	0.57	0.734	0.688
The results were analysed by Students't' test and compared with control for coming to conclusion. The results are expressed as Mean ± standard error of the mean. ***P<0.0001.					

TABLE 15

EFFECT OF *CARISSA CARANDAS* METHANOLIC EXTRACT ON CASTOR OIL INDUCED FECAL OUTPUT

Treatment	Fecal output							
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	Total fecal output in 6 hrs	Avg fecal output in 6 hrs
Saline (2 ml/kg, ip)	1.15 ± 0.201	1.045 ± 0.157	0.26 ± 0.116	0.047 ± 0.029	1.388 ± 0.458	0.406 ± 0.211	4.297 ± 0.608	0.715 ± 0.101
Atropine (3 mg/kg, ip)	0.068 ± 0.031***	0.617 ± 0.229	0.048 ± 0.046	0.403 ± 0.202	0.0***	1.143 ± 0.377	2.28 ± 0.39**	0.422 ± 0.077
CCME (50 mg/kg, po)	1.027 ± 0.109	0.71 ± 0.114	0.19 ± 0.035	0.105 ± 0.048	0.1 ± 0.044*	0.48 ± 0.27	2.612 ± 0.107***	0.743 ± 0.03
CCME (100 mg/kg, po)	0.865 ± 0.081	0.465 ± 0.0806*	0.248 ± 0.097	0.91 ± 0.096***	0.647 ± 0.232	0.105 ± 0.048	3.24 ± 0.273*	0.927 ± 0.079
CCME (200 mg/kg, po)	0.322 ± 0.111*	0.273 ± 0.098*	0.8 ± 0.253	0.205 ± 0.1	0.688 ± 0.241	0.0***	2.288 ± 0.356**	0.654 ± 0.101

The results were analysed by Students't' test and compared with control for coming to conclusion. The results are expressed as Mean ± standard error of the mean. *P<0.01, **P<0.001, ***P<0.0001.

TABLE 16

EFFECT OF *CARISSA CARANDAS* ROOT EXTRACT ON GI MOTILITY INDUCED BY CASTOR OIL

Treatment	Total Intestinal transit of charcoal meal	% Inhibition of charcoal meal transit
Control (Saline 2 ml/kg, ip)	91.87 ± 1.975	----------
Atropine	50.35 2.91***	44.1
CCME (50 mg/kg, po)	36.28 ± 2.98***	60.5
CCME (100 mg/kg, po)	24.85 ± 1.36***	72.95
CCME (200 mg/kg, po)	75.475 ± 2.31**	17.86

The results were analysed by Students't' test and compared with control for coming to conclusion. The results are expressed as Mean ± standard error of the mean. ***P<0.0001 and **P<0.001.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

In summary, the *Carissa carandas* has been investigated in detail for its pharmacognostic and phytochemical characterization and its possible medicinal applications for diarrhea, ulcer CNS depression, stress and adaptogenic activity by using preclinical animal models.

The results of microscopic and morphological investigations along with the organoleptic characteristics of *Carissa carandas* root preparations gives an important information on its methods of botanical standardization, authentication and powder characteristics for evaluating its adulteration. The standardization of the root was carried out by total ash value, acid insoluble ash value, water and alcoholic extractive values. The crude extracts of *Carissa carandas* root uniform powder obtained by successive solvent extraction was screened blindly in swiss albino mice for possible biological activity.

The important part of the present investigation was phytochemical investigation, in the present study after successive solvent extraction of *Carissa carandas root* uniform powder, the extracts undergone biological evaluation to know the biologically active constituents present in root preparation. The results of biological investigation has shown benzene extract possess more potent antibacterial and antifungal activity, which was also reflected in methanolic extract but less potent. The blind screening of all the extracts while acute toxicity evaluation indicated the petroleum ether extract has CNS stimulant property, hence it was considered for evaluating its antidepression and adaptogenic properties. However, qualitative chemical investigation shown the presence of only steroid, therefore it was not considered for phytochemical investigation. The preliminary and in detail spectral analysis of benzene extract shown the presence of unsaturated fatty acid, saturated fatty acid and aromatic amides as

important phytoconstituens which could be responsible for potent antibacterial and anti-fungal activity. The methanolic extract's chemical investigation shown the presence of aromatic amides, may be responsible for anti-fungal, anti-diarrheal, anti-ulcer, regulating intestinal and gall bladder movement.

It is believed that saturated and unsaturated fatty acids and aromatic amides produce increased membrane permeability thereby leading to antimicrobial effect. The fatty amides are proposed to produce their effect by conjugation with surface membrane proteins and peptides thereby destabilizing the membranes and producing antimicrobial effects. It is also believed that the aromatic amides undergoes metabolism and converting into active amine/amides and producing actions similar to biogenic amines/amides with higher lipophilicity therefore, producing more prominent effects on CNS due to their ability to cross BBB.

We conclude, from the present study that the phytoconstituents saturated and unsaturated fatty acids and aromatic amides obtained in the present study could be useful in bacterial and fungal infectious conditions. They could also be useful in depression, stress, diarrhoea, ulcer and biliousness. However, further studies on 2D NMR and 13C NMR are required to elucidate the complete structure of isolated phytochemicals. The isolated and characterized compounds from *Carissa carandas* root methanolic and benzene extracts may need lead optimization for improving their efficiency and potency. We hereby warn that further studies are going on in our laboratory in this direction.

BIBLIOGRAPHY

BIBLIOGRAPHY

Agarwal SK, Verma S, Singh SS, Kumar S, Keshri G, 1999. Antifertility chromene from Blepharispermum subsessile. Fitoterapia, 70, 435-437.

Aikman L, 1974. We, the people; the story of the United States Capitol, its past and its promise. National Geographic Magazine, 420.

Akerele O, 1993. Nature's Medicinal bounty: Don't throw it. World Health Forum, 14 (4), 390-395.

Alexander F, Chu-Kung, Kristen N, Bozzelli NA, Lockwood MV, 2003. Effect of Fatty Acid Conjugation on Antimicrobial Peptide Activity. Materials Research Society, 506.

Ammon HV, 1974. Thomas PJ, Phillips S. Effect of oleic and recinoleic acid on net jejunal water and electrolyte movement. J Clin Inves, 53: 374-379.

Aranda-Michel J, Gianella RA, 1999. Acute diarrhoea: A practical review. Am J Med, 106: 670-676.

Asandberg F, 1975. The significance of adptogenic substances for the organism. Proceedings of Symposium of Gerontology; Lugano.

Bannerman RH, 1982. Traditional medicine in modern health care. World Health Forum, 3 (1), 8-13.

Basanagouda MP, Khatib N, Boreddy ST, 2004. L-arginine-nitric oxide pathway modulates morphine-induced inhibition of gall bladder emptying in mice. J. Pharmacy Pharmacology Rapid Communication, 36, 262-264.

Bhal BS, Bhal A, 2004. Text book of Organic Chemistry, CBS Publishers, New Delhi, 202-218.

Bhattacharya SK, Bhattacharya A, 2000. Adptogenic Activity of siotone,apolyherbal formulation of Ayurvedic rasayanas. Indian J Exp Biol. 38 (2), 119-28.

Bhatwadekar AD, Chintawar SD, Logade NA,Somani RS, Kasture SB, 1999. Antistress Activity of Butea monosperma Flowers. Indian J Pharmacol. 31, 153-155.

Borris RP, 1996. Natural products research: Perspectives from a major pharmaceutical company. Journal of Ethnopharmacology, 51, 29-38.

Brekhman II, Dardymov IV, 1969. New Substances of plant origin which increase non specific resustance. Ann Rev Pharmacol. 9, 419-430

Byrnes DJ, Borody T, Daskalopouloues G, Boyle M, 1981. Cholecystokinin and gall bladder contraction effect of CCK infusion. Peptides, 2 (Supp 2): 256-62.

Chakraborti KK, Handa SS, 1989. Natural products and plants as liver protecting Indian Drugs 27, 19-24.

Chatwal GR, Anand SK, 1979. Instrumental analysis. First Edition, CBS Publisher New Delhi. 2.599-2.600.

Chawla AS, Handa SS, Sharma AK, Kaith BS, 1992. A lignan from Vitex negundo seeds. Phytochemistry, 31 (12), 4378-4379.

Chevallier A, 2001. In, Herbal remedies handbook: A natural approach tohealing common and chronic ailments. Darling Kindersly Limited, London, 10-17.

Chitme HR, Kaushik S, Chandra R, 2004. Studies on anti-diarrhoeal activity of Calotropis gigantea R.Br . in experimental animals. J Pharm Pharmaceut Sci., 70 (1), 70-75.

Ciereszko A, Dabrowski K, 2000. In vitro effect of gossypol acetate on yellow perch (Perca Flaescens) spermatozoa. Aquatic Toxicology, 49, 181-187.

Colmeiro-lafforet C, 1962. Contraception of vegetal origin. Revista Esponola de Obstetriciay Ginecologica, 21, 179-184.

Comley JC, 1990. New macrofilaricidal leads from plants. Tropical Medicine and Parasitology, 41 (1), 1-9.

Compadre CM, Pezzuto JM, Kinghorn AD, Kamath SK, 1985. Hernandulcin: an intensely sweet compound discovered by review of ancient literature. Science, 227, 417-419.

Cooper JR, Bloom FE, Roth RH, 2003. The biochemical Basis of Neuropharmacology. Oxford University Press , UK : London, 380.

Copper and Gunns, 2005. Tutorial Pharmacy, First Edition, 251-252.

Cordell GA, Farnsworth NR, 1976. Process for the preparation of alkaloids of the leurosine type. Heterocycles 4, 393-427.

De Pasquale A, 1984. Pharmacognosy: The oldest modern science. Journal of Ethnopharmacology, 11, 1-6.

De Smet PA, 1997. The role of plant derived drugs and herbal medicines in health care. Drugs, 54 (6), 801-840.

Dobrakovova M, Kvetnansky R, Oprasalova Z, Macho L, 1990. The Effect of chronic stress on the sympathetic – adrenomedullary system. Brantislavske Listy. 91, 587-592.

Easu K, 1964. Plant anatomy; John Wiley and Sons; New York. 767. Easu K, 1979. Anatomy of Seed plants; John Wiley and Sons; New York. 550.

Eiswirth NA, Smith DE, Wesson DR, 1972. Current Perspective on cocaine use in America, J. Psychodelic Drugs, 5, 153-157.

Farnsworth NR, 1980. The development of pharmacological and chemical research for application to traditional medicine in developing countries. Journal of Ethnopharmacology, 2 (2), 173-181.

Farnsworth NR, 1985. Setting priorities for cancer control programmes. World Health Forum 4, 76.

Farnsworth NR, 1990. The role of ethnopharmacology in drug development. Ciba Foundation Symposium, 154, 2-11.

Farnsworth NR, Cynthia JK, 1981. An approach utilizing information from traditional medicine to identify tumor-inhibiting plants. J. Ethnopharmacol, 3 (1), 85-99.

Farnsworth NR, Soejarto DD, 1985. Potential consequ-ences of plant extinction in the United States on the current and future avaibility of prospecting drugs. Econ. Bot., 39, 231–240.

Fontaine-O, 1988. Diarrhoea and treatment. Lancet, 28, 1234-1235.

Gaginella TS, Stewart JJ, Olson WA, Bass P, 1975. Actions of ricinoleic acid and structurally related fatty acid on the gastro-intestinal tract II. Effects on water and electrolyte absorption in vitro. J Pharmacol Exp Ther, 195, 355-361.

Gamble JS, 1935. Flora of the Presidency of Madras Vol. I II & III Botanical Survey of India, Calcutta, India.

Gbile ZO, Adesina SK, 1987. Nigerian flora and its pharmaceutical potential. J. Ethnopharmacol 19 (1), 1-16.

Gregory SK, 2001. Rhodiolarosea: A possible Plant Adaptogen. Alt Med Rev. 6(300), 293-302.

Hamburger M, Hostettman K, 1991. Bioactivity in plants: The link between phytochemistry and medicine. Phytochemistry, 30 (12), 3864-3874.

Handa SS, 1971. Pharmacos. 16, 53.

Handa SS, 1985. Punjab University Alumni. P 4.

Handa SS, Gupta SK, Vasisht K, Phillipson JD, 1984. Quinoline alkaloids from Anthocephalus chinensis. Planta Med. 50 (4) 358.

Handa SS, Kapoor VK, 1989. Pharmacognosy, 2 Edition, Vallabh Prakashan, New Delhi 183.

Handa SS, Kinghorn AD, Cordell GA, Farnsworth NR, 1983. Plant Anticancer Agents. XXVI. Constituents of Peddiea fischeri. J. Nat. Prod. 46, 248-250.

Handa SS, Kinghorn AD, Cordell GA, Farnsworth NR, 1983. Plant Anticancer Agents XXV. Constituents of Soulamea soulameoides. J. Nat. Prod. 46, 359-364.

Handa SS, Sarangi SR, Kanitkar UK, 1968. J. Univ. Poona Sci. Tech. 34, 51.

Handa SS, Sharma A, 1989. In Research and Development of Indigenous Drugs. Institute of History of Medicine and Medical Research. 147.

Handa SS, Sharma A, 1990. Hepatoprotective activity of. andrographolide from Andrographis paniculata against. carbon tetrachloride. Indian J. Med.Res. 92, 276 and 284.

Ieven M, Vanden BDA, Mertens F, Vlientinck A, Lammens E, 1979. Screening of higher plants for biological activities. I. Antimicrobial activity. Planta Medica, 36 (4), 311-321.

Jain SK, Chitme HR, Chandra R. 2005. Antimalerial of New herbal formulation. Indian J Pharm Sci. 1, 101-103.

Johnasen DA, 1940. Plant Microtecnique; Mc Graw Hill Book Co; New York; 523. O Brien TP, Feder N, Mc cull ME, 1964. Polychromatic staining of Plant cell walls by Touidine Blue –O. Protoplasm, 59, 364-373.

Kabara et al., 1979. Toxicological, Bateriocidal and Fungicidal Properties of Fatty Acids and Some Derivatives. J. Am. Oil Chemists Socy, 760A-767A.

Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP, 1972. Fatty Acids and Derivatives as Antimicrobial Agents. Antimicrob Agents Chemother. 2 (1), 23–28.

Kabara JJ, Vrable R, Lie Ken Jie MSF, 1977. Antimicrobial lipids: Natural and synthetic fatty acids and monoglycerides. Lipids, 12 (9), 753-759.

Kalsi PS, 2002. Spectroscopy of organic compounds. First Edition, CBS Publisher New Delhi.

Kartikar KR, Basu LM, Basu MD, 1984. Indian Medicinal Plants. Allahabad, India, 1546-1548 .

Kawangh R, 1963. Analytical microbiology, Academic Press, London, 125-141.

Khandelwal KR, 1994. Practical pharmacognosy. Twelth Edition pp; 157-159.

Kinghorn AD, 1982. Pharm. International. 3, 362.

Kithara T, Koyama N, Matsuda J, Atsuda J, Aoyama Y, Hirakata Y, Kamihira S, Kohno S, Nakashima M, Sasaki H, 2004. Antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant Staphylococcus aureus. Biological & Pharmaceutical Bulletin, 27 (9), 1321-1326.

Kleijnen J, Knipschild P, 1992. Ginkgo biloba. Lancet, 340 (8833), 1474.

Labadie RP, 1986. Problems and possibilities in the use of traditional drugs. Journal of Ethnopharmacology, 15, 221-230.

Levy G, 1982. Gastrointestinal clearance of drugs with activated charcoal. New Eng J Med, 307, 676-678.

Lobo E, Kulkarni RD, Desai RR, 1975. Special Pharmacology of Geriforte. Probe, 4266.

Malave A, Yim GKW, 1992. Effects of chronic morphine on biliary tract responses to cholecystokinin octapeptide in female guinea pigs. Life Sci. 5, 695-700.

Maseko DN, Hoff W, 1986. World Health Forum 7, 412.

Meyer HS, Eldrege JD, (editors), 1998. Books, journals, new media. Journal of American Medical Association, 279 (1), 85-89.

Mukerjee T, Bhalla N, Aulakh GS, Jain HC, 1984. Herbal drugs for urinary stonesÐliterature appraisal. Indian Drugs, 221, 224-228.

Nadkarni AK, 1954. Dr K. M. Nadkarni's Indian Materia Medica, Bombay, Popular Book Depot, 266.

Nagraj HS, Jaganathan PS, 1999. Influence of different types of stress on slected cardiovascular parameters in rats. Indian J Physiol Pharmacol. 43 (3), 296-303.

Olitzky, 1965. Antimicrobial Properties of a Propylene Glycol Based Topical Therapeutic Agent. J. Pharmaceutical Sci., 787-788.

Park K, 2000. Park's Text book of Preventive and Social Medicine. Banarsidas Bharat Publishers, Jabalpur, 122-175.

Philipson JD, 1994. Natural products as drugs. Transactions of the Royal Society of Tropical Medicne and Hygiene, 88 (1), S17-S19.

Phillipson JD, 2001. Phytochemistry and medicinal plants. Phytochemistry, 56, 237-243.

Phillipson JD, Thomas OO, Gray AI, Sariyar G, 1981. Alkaloids from Papaver armeniacum, P. fugax and P. tauricola. Planta Med. 41(2), 105-118.

Pitman DL, Ottenweller JE, Natlson BH, 1988. Plasma corticosterone levels during repeated presentation of two inensities of restraint stress; chronic stress and habituation. Physiol Behav. 43, 47-56.

Poller WG, Hollister LE, 2004. Antidepressant Agents. IN: Katzung BG eds. Basic And clinical pharmocolgy. New Delhi: Mc Graw Hill, 482-496.

Quezada N, 1975. Traditional contraceptive and abortive methods. Anales de Antopologia, 12, 223-242.

Rates SMK, 2001. Plants as a source of drugs. Toxicon, 39, 603-613.

Reisine T, Pasternak G, 1996. Opioids analgesics and antagonists; morphine and related opioids agonist. In: Goodman LS, Gilman A, Limbrid LE, editors. The Pharmacological Basis of Therapeutics. 9th eds. New York: Mac Millan; 532.

Rios JL, Recio MC, Giner RM, Manez S, 1996. An update: review of saffron and its active constituents. Phytotherap Res, 10, 189-893.

Sahgal VK, Sood NK, 1975. Geriforte;An indigenous geriatric tonic. In hyperlipidaemia. Probe., 4, 292.

Sass JE, 1940. Elements of Botanical Microtecnique. Mc Graw Hill Book Co; 222.

Henry AN, Kumari GR, Chitra V, 1987. Flora of Taminadu , India.Vol. I, II & III Botanical Survey of India, Southern Circle, Coimbatore, India .

Schiebinger L, 2000. Exotic abortifacients: The global politis ofplants in the 18th century. Endeavour, 24 (3), 117-121.

Schimer RE, 2004. Modern methods of Pharmaceutical analysis. Second Edition Volume I, 25-27.

Sely H, 1976. The Psychosocial environment and psychosomatic diseases. Devi L, Oxford University Press (NY), 76-78.

Sharma AK, Pushpaangadan P, Chopra CL, Rajsekharan S, Saradaamma L, 1989. Adaptogenic activity of seeds of Trichopus Zelyanicus gaertin,the ginseng of Kerala. Anc Sci Life, 8, 212-219

Shay H, Sun DCH, Gruenstein M, 1954. A qualitative method for measuring spontaneous gastric secretion in the rat. Gastroenterology, 26, 906-913.

Silverstein B, Morriss B, 2004. Spectrometric identification of organic compounds. 181-186.

Singh B, Saxena AK, Chandan BK, Gupta DK, Bhutani KK, Anand KK, 2001. Adptogenic Activity of a novel,withanolide-free aqueous fraction from the roots of Withania somnifera Dun. Phytother Res. 15(4), 311-318.

Singh N, Nath R, Mishra N, Kholi RP, 1978. An experimentalevaluation of antistress effects of Geriforte (an Ayurvedic drug).Quart J crud Drug Res. 3, 125-133.

Singh Y, 1992. Kava: An Overview. J Ethnopharmacol, 37, 13-45.

Snyder JD, Merson MH, 1982. The magnitude of the global problem of acute diarrhea disease: A review of active surveillance of data. Bull WHO, 60, 605-613.

Sreemantulal S, Nammi S, Koanukondal R, Koppulal S, Boini KM, 2005. Adaptogenic nootropic activities of extract of Vitis vinifera (grape seed):an experimental study in rat model. BMC Coml Alt Med, 5 (1), 1472-6882.

Staritiz M, 1988. Pharmacology of the Spintcher of Oddi. Endoscopy, 20 (Supp 1); 171-174.

The Ayurvedic Pharmacopoeia of India, 1999. Part 1, Vol, ll, 1st Edn, Govt of India, Ministry of Health and Family Mc Graw Hill Book co; New York ; 191-192. Evans WC, 1996.

The Wealth of India, 1992. Vol 3, Revised Edition, Publication and Information Directorate, New Delhi: CSIR; 1102-1104.

Trease and Evans, Pharmacognosy 14th Edn. WB saunders Company Ltd, Londen, 1996, 194-196.

Turner DM, 1996. Natural product source material use in pharmaceutical industry: The glaxo experience. Journal of Ethnopharmacologyy, 51, 39-44.

Vagh VT, Kapadia HD, Pavri DN, 1975. Clinical evaluation of an indigenous geriatric tonic. Probe. 4, 292.

Valsecchi B, Toson G. 1982. Mouse gall bladder emptying by egg yolk: a possible new rapid method for anti spasmodic activity evaluation of in vivo. J Pharmacol Methods, 7, 193-195.

Venkatesan N, Thiyagarajan V, Narayanan S, Arul A, Raja S, Gurusamy S, Vijaya Kumar, Rajarajan T, Perianayagam PB. 2005. Anti-diarrhoeal potential of asparagus racemosus wild root extracts in laboratory animals. J Pharm Pharmaceut Sci. 8(1), 39-46.

Willard M, Dean S, 2002. Instrumental methods of analysis. Sixth Edition, 565-567.

Yu GD, 1982. Medicinal plants used for abortion and childbirth in Eastern Africa. Zhong Yao Tong Bao, 7 (5), 6-7.

LIST OF PUBLICATIONS AND PRESENTATIONS

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1. The research article entitled 'Adaptogenic and anti-stress activity of *Carissa carandas* in rats' published in Current Journal of Pharmaceutical Research, 2006 1(2), 64-68.
2. The research article entitled 'Preliminary study on antidiarrheal activity of *Carissa carandas* Linn root extract' accepted for publication in Journal of Herbal Pharmacotherapy, 2007.
3. The research article entitled 'Antibacterial and phytochemical evaluation of *Carissa carandas* Linn root extract' communicated to Fitoterapia for publication in 2007.
4. The research article entitled 'Antifungal and phytochemical evaluation of *Carissa carandas* Linn root extract' communicated to Phytotherapy Research for publication in 2007.
5. The research article entitled 'Pharmacognostic and phytochemical study *Carissa carandas* Linn root extract' communicated for publication in International Journal of Crude Drugs, 2007.
6. The research article entitled 'Antidiarrheal activity of *Carissa carandas* Linn root extract' presented at National Seminar on Emerging Trends in Ethnopharmacology, Bagalkot Karnata-2005.
7. The research article entitled 'Antifungal activity of *Carissa carandas* Linn' presented at National Seminar on Emerging Trends in Synthetic aspects of medicinal chemistry, Warangal AP-2005.
8. The research article entitled 'Antimicrobial activity of *Carissa carandas* Linn' presented at XXXIX Annual conference of Indian Pharmacological Society, Jaipur -2006.

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Anti-Stress And Adaptogenic Activity Of Karanda Root Extract

Mallikarjun Malipatil[1], H. R. Chitme[2*], Ramesh Chandra[3]

1: Karnataka College of Pharmacy, Bidar, Karnataka, India.

2: Dept of Pharmacology, H. S. K. College of Pharmacy, Bagalkot, Karnataka, India.

3: Ambedkar Center for Biomedical Research, Delhi University, India.

Abstract: *Plant adaptogens cause our physiology to begin the adaptation process to stress. When a stressful situation occurs, consuming adaptogens generates a degree of generalized adaptation that allows our physiology to handle the stressful situation in a more resourceful manner. The Carissa Carandas linn commonly known as karanda, is common throughout India and is valued in intermittent fever, diarrhea, inflammation etc. In the present study the petroleum ether root extract of C. Carandas was evaluated for antidepression and antistress property. Adaptogenic activity was evaluated by using swimming model and duration of swimming was considered for evaluation. Restrain stress model was used for antistress activity and ulcer index was considered for evaluation. The small dose (100 mg/kg, po) and maximum dose (400 mg/kg, po) significantly increased the duration of swimming, whereas the moderate dose failed to produce significant change in duration of swimming. 100 mg/kg extract also reduced the stress as ulcer index was significantly decreased than compare to control group ($P<0.01$). We conclude from the present study that the small dose of the extract significantly produce antidepressant and antistress activity than compare to control groups and shall have beneficial effects as adaptogen. However, the study is on in our laboratory to find out its phytochemical nature and mechanism of action and hence is warned.*

Keywords: Adaptogen, anti-fatigue, antistress, *Carissa carandas*, Karanda

***Corresponding Address:**
H. R. Chitme
Dept of Pharmacology
H. S. K. College of Pharmacy
Bagalkot-587101, Karnataka-India
hrchitme@rediffmail.com
Tel: +91-8354-220008
Fax: +91-8354-225102

INTRODUCTION:

Stress represents a reaction of the body to a stimulus that tends to alter its normal physiological equilibrium or homeostasis and has been defined as a nonspecific response of the body to any demand imposed on it (Sreemantulal et al., 2005) and elicits various endocrinal and visceral changes such as changes in plasma cortisone and gastric mucosal integrity (Bhatwadekar et al., 1999). To successfully combat stress and stressful situations, adaptation is required. Adaptation might be best thought of as the ability to be exposed to a stressor, while responding with either decreased or no characteristic hormonal perturbations. Adaptation also implies being prepared to and capable of rapidly reassuming homeostasis after the stressor is withdrawn (Gregory, 2001). An adaptogen produces a non-specific response in an organism; i.e., an increase in power of resistance against multiple stressors including physical, chemical, or biological agents. An adaptogen also has a normalizing influence on physiology, irrespective of the direction of change from physiological norms caused by the stressor. However it is incapable of influencing normal body functions more than required to gain non-specific resistance (Brekhman and Dardymov, 1969). Since the introduction of adaptogens, several plants have been investigated, which were once used as tonics due to their adaptogenic and rejuvenating properties in traditional medicine. The drugs of plant origin are gaining increasing popularity and are being investigated for remedies of a number of disorders including antistress activity (Sreemantulal et al., 2005). Plant adaptogens cause our physiology to begin the adaptation process to stress. When a stressful situation occurs, consuming adaptogens generates a degree of generalized adaptation that allows our physiology to handle the stressful situation in a more resourceful manner (Brekhman and Dardymov, 1969).

The *Carissa carandas L.* commonly known as karanda is native and common throughout much of India, Burma, and Malacca and area of Ceylon; is rather commonly cultivated in these areas as a hedge and for its fruits and the fruit is marketed in villages. In Asia, the ripe fruits are utilized in curries, tarts, puddings and chutney. When only slightly underripe, they are made into jelly. Green, sour fruits are made into pickles in India. With skin and seeds removed and seasoned with sugar and cloves, they have been popular as a substitute for apple in tarts. British residents in India undoubtedly favored the karanda as being reminiscent of gooseberries (The Wealth of India, 1992; Kartikar et al., 1984; Nadkarni, 1954).

The unripe fruits are used medicinally as an astringent. The ripe fruits is taken as an antiscorbatic and remedy for biliousness. The leaf decoction is valued in cases of intermittent fever, diarrhea, oral inflammation and earache. The root is employed as a bitter stomachic and vermifuge and it is an ingredient in a remedy for itches. The roots contain salicylic acid and cardiac glycosides causing a slight decrease in blood pressure. Also reported are carissone; the D-glycoside of β-sitosterol; glucosides of odoroside H; carindone, a terpenoid; lupeol; ursolic acid and its methyl ester; also carinol, a phenolic lignan. Bark, leaves and fruit contain an unnamed alkaloid (The Wealth of India, 1992; Kartikar et al., 1984; Nadkarni, 1954).

The purpose of the present study is to evaluate adaptogenic and antistress activity of petroleum ether extract of *C. carandas* root in rats.

MATERIAL AND METHODS:

Plant Preparation:

The root of *Carissa carandas* was collected, dried under shade and cut into pieces, crushed into powder then passed through sieve #40 to obtain uniform particles and extracted successively with the solvents of increasing polarity Petroleum Ether, Benzene, Chloroform, Ethyl acetate, Methanol and water, by successive solvent extraction method (Jain et al., 2005). For each solvent 12 hrs cycle of extraction was carried out and concentrated by flash evaporator, dried at low temperature in freeze dryer under vacuum. Henceforth the extract means petroleum ether extract of *Carissa carandas* root extract. The extract was reconstituted in propylene glycol as a solvent.

Adaptogenic Activity:

Adaptogenic activity was evaluated by following Sharma, et al (1989) method with little modification; briefly, the animals were grouped into four, six animals in each group, one is control and other three groups were treated, control group received propylene glycol (1% body weight, p.o.) and other treated groups received extract of 100, 200 and 500 mg/kg body weight (p.o.) respectively. The drug was administered 1 hour earlier to animals forced for swimming. Wistar albino rats of body weight 200 to 250 gm were individually subjected to swim in a vertical plexiglass cylinder (height: 40 cm diameter: 18 cm, containing 15 cm of water). Duration of swimming was recorded for a period of 14 days and compared with control group.

Antistress Activity:

Group of six female wistar rats per dose of test drug and for controls weighing 200-250 gm were used. The extract was administered continuously for 14 days time and animals were restrained in

polypropylene rats restrainer in prone position for a period of one hour daily lasting for 15 days. The antistress activity was evaluated by using ulcer score and ulcer index and treated groups were compared with control. Finally animals were sacrificed, stomach was removed, fixed on cork plate and the number and severity of ulcers were registered with a lens using the following score- 0=no ulcer; 1=superficial ulcer; 2=deep ulcer; 3=perforation. The ulcer index was calculated by the formula, $U_I = U_S + U_N + U_P X\ 10^{-1}$ where, Un = average number of ulcers per animal, Us = average of severity score and Up = percentage of animals with ulcers (Nagaraj and Jaganathan, 1999).

Statistical Evaluation:

The results are expressed as mean ± standard error of mean (S.E.M.). The results obtained from this study were evaluated by students 't' test. The test doses were compared with control group and ulcer index was calculated along with scoring the severity of ulcer.

RESULT:

Adaptogenic Activity:

Table 1 shows that, the administration of maximum dose of extract produced a significant ($P<0.01$) increase in the swimming duration as compared to the control from day 1 to till the completion of study. A dose of 200 mg/kg did not produce significant change in duration of swimming, and mean duration of swimming time was without significant difference than compared to control group. The small dose of the extract 100 mg/kg gradually improved the performance and significantly increased the duration of swimming on 14 day of study than compare to control.

Table-1: Adaptogenic Activity Of Carissa Carandas Linn Using Swimming Model

Treatment	1 Day	2 Day	4 Day	7 Day	14 Day
Control	1.1 ±0.14	1.30 ± 0.07	1.39 ± 0.15	1.14 ± 0.1	1.01± 0.18
(1% BW Propylene glycol)	0.31± 0.06	0.5 ± 0.074	1.63 ± 0.17	1.18 ± 0.48	2.70 ± 0.4**
Extract (100 mg/kg)	0.85 ± 0.08	0.86 ± 0.03	1.14 ± 01.5	1.08 ± 0.16	1.7 ± 0.37
Extract (200 mg/kg)	2.18 ± 0.33*	1.58 ± 0.47	2.1 ± 0.36**	2.89± .48**	3.1± 0.6**
Extract (500 mg/kg)					

The extract was administered 1 hr prior to forcing the rats for swimming. Results are presented as Mean ± SEM and analysed by students t test.*P<0.05, **P<0.01.

Antistress Activity:

Table 2 shows that, the extract pretreatment on restrained rats produced a significant reduction in severity of ulcer as well as ulcer index. All the doses selected in the study significantly ($P<0.5$ and $P<0.01$) reduce the severity of ulcer by reducing the ulcer score. However, 100 mg/kg dose of the extract decreased the ulcer index but moderate and high dose of extract failed to decrease the ulcer index than compared to control. The 200 mg/kg and 500 mg/kg dose of the extract also failed to overcome the hemorrhage and to reduce the total mucosal area affected in restrained stress.

Table-2: Anti-Stress Activity Of Carissa Carandas Linn Using Restrain Stress Model

Treatment	Ulcer Index	Ulcer Score
Control	1.08 ± 0.01	3.3 ± 0.3
Extract 100 mg/kg	0.86 ± 0.07**	1.7 ± 0.6**
Extract 200 mg/kg	1.07 ± 0.07	2.3 ± 0.6*
Extract 500 mg/kg	1.07 ± 0.06	2.4 ± 0.8*

The extract was administered 1 hr prior to restraining the rats. Results are presented as Mean ± SEM and analysed by students t test. *P<0.05, **P<0.01.

DISCUSSION:

Stress is one of the silent killing disorder, which is also associated with several neurochemical, psychobehavioural, physiological, pathological, pathophysiological and biochemical changes (Dobrakovova et al., 1990). Stress, age and behaviour characteristics are considered to be risk factors for disturbances of the cardiovascular system in animal and man. It has been suggested that chronic stress can contribute to the development of exacerbation of cardiovascular dysfunction (Pitman et al, 1988). Exposure to various stressful stimuli triggers the activation of hypothalamo pituitary adrenal (HPA) axis and the sympathetic adrenomedullary system. There is considerable evidence showing that the HPA response is progressively reduced after repeated exposure to the same stressor. This

phenomenon called adaptation was shown to occur following exposure to various chronic intermittent stressors such as physical restraint (Pitman et al., 1988; Selye 1976; Asandberg, 1975). Stress has been suspected to be one of the mechanisms leading to disease under certain circumstances (Nagaraj and Jaganathan, 1999), several studies demonstrated that certain plant extracts and some of the glycosides obtained from these produce a state of non-specifically increased resistance in animals and human beings (Lobo et al., 1975). Adaptogens are known to induce cellular regeneration, prevent arteriosclerosis, increase hormone utilization enhance protein and carbohydrate metabolism and produce reduction in serum lipid (Sahgal and Sood, 1975; Vagh et al., 1975). Besides it has been proved useful clinically by producing a feeling of well-being, increasing mental activity, lessening fatigue, increasing appetite and sexual function in the ageing (Bhattacharya et al., 2000; Singh et al., 2001; Singh et al., 1978). The stimulant action of Coca leaves was explained as due to the presence of the alkaloid cocaine or and their metabolites (Eiswirth et al., 1972).

All parts of the plant are reputed in indigenous medicine. The roots are credited with bitter, stomachic and anthelmintic properties. The alcoholic extract of roots exhibited cardiotonic activity and a deep and prolonged blood pressure lowering effect. The roots contain carissone, carindone, carinol, sitosterol. An amorphous water-soluble polyglycoside fraction possessing significant cardiac activity has also been isolated; the hydrolysis of the fraction gave odoroside H, digitoxigenin, glucose and D-digitalose. The cardiotonic activity of water soluble fraction has been attributed to the presence of glucosides of odoroside H. Presence of alkaloids is also reported in root and stembark (The Wealth of India, 1992).

In the present study we evaluated the adaptogenic property of petroleum ether extract of *Carissa carandas* root. We used the swimming endurance model for preliminary evaluation of its possible adaptogenic property and the period of swimming was considered as the parameter. The small dose and maximum dose of the extract selected for study has shown dramatic effects than compare to higher doses. The small dose of extract progressively improved the performance till the 14 days of study, however the higher dose significantly increased the performance from day 1 of the study and continued till the last day of study.

The increased performance of the extract indicates its possible presence of adaptogenic properties in it. Therefore, a rational approach was made to evaluate its antistress properties. For this, restraint stressful situation was induced in animals. Improved swimming duration and prevention of stress-induced ulcers indicate the anti-stress properties of *C. carandas*. These activities could be mediated due to the presence of flavones, terpenoids, alkaloids and glycosides.

We conclude from the present study that the low dose of the extract is safe and may be used safely for the improvement of performance because of presence of anti-stress properties. In this study the extract reduced total ulcer score significantly and dose dependently but the moderate and maximum dose failed to reduce ulcer index significantly than compare to control. The herbal extract at minimum dose may be safe and beneficial in coping up the stressful condition and to improve and maintain vitality, increased efficiency and immunity of the body.

REFERENCES:

1. Asandberg, F., (1975) The significance of adaptogenic substances for the organism. Proceedings of Symposium of Gerontology; Lugano.
2. Bhattacharya, S. K., Bhattacharya, A. and Chakrabarti, A. (2000). Adaptogenic activity of Siotone, a polyherbal formulation of Ayurvedic rasayanas. Indian J. Exp. Biol. 38, 119-128.
3. Bhatwadekar, A. D., Chintawar, S. D., Logade, N. A., Somani, R. S., Kasture, V. S. and Kasture, S. B. (1999). Antistress Activity of Butea monosperma Flowers. Indian J. Pharmacol. 31, 153-155.
4. Brekhman II and Dardymov IV. (1969). New substances of plant origin which increase nonspecific resistance. Ann. Rev. Pharmacol. 9, 419- 430.
5. Dobrakovova, M., Kvetnansky, R., Oprasalova, Z. and Macho, L. (1990). The effect of chronic stress on the sympathetic-adrenomedullary system. Brantislavske Leakarske Listy. 91, 587-92.
6. Eiswirth, N. A., Smith, D. E. and Wesson, D. R. (1972). Current Perspectives on cocaine use in America. J. Psychodelic Drugs 5, 153-157.
7. Gregory, S. K., (2001). Rhodiola rosea: A Possible Plant Adaptogen. Alt. Med. Rev. 6, 293-302.
8. Jain, S. K., Chitme, H. R. and Chandra, R. (2005). Antimalarial activity of new herbal formulation. Indian J. Pharm. Sci. 1, 101-103.
9. Kartikar, K. R., Basu, L. M. and Basu, M. D. (1984). Indian Medicinal Plants. 2 Ed. Allahabad, India; pp 1546-1548.
10. Lobo, E., Kulkarni, R. D. and Desai, R. R. (1975). Special Pharmacology of Geriforte. Probe. 4, 266.

11. Nadkarni, A. K, (1954). Dr K. M. Nadkarni's Indian Materia Medica. Popular Book Depot, Bombay; pp 266.
12. Nagaraj, H. S. and Jaganathan, P. S. (1999). Influence of different types of stress on selected cardiovascular parameters in rats. Indian J. Physiol. Pharmacol. 43, 296-304.
13. Pitman, D. L., Ottenweller, J. E., Natlson, B. H. (1988). Plasma corticosterone levels during repeated presentation of two intensities of restraint stress; chronic stress & habituation. Physiol. Behav. 43, 47-56.
14. Sahgal, V. K. and Sood, N. K. (1975). Geriforte; An indigenous geriatric tonic in hyperlipidaemia. Probe. 4, 277.
15. Selye, H. (1976). The Psychosocial environment and psychosomatic diseases. Devi L, editor, Oxford University Press (NY).
16. Sharma, A. K., Pushpangadan, P., Chopra, C.L., Rajsekharan, S. and Saradaamma, L. (1989). Adaptogenic activity of seeds of Trichopus zeylanicus gaertin, the ginseng of Kerala. Anc. Sci. Life. 8, 212-219.
17. Singh, B., Saxena, A. K., Chandan, B. K., Gupta, D. K., Bhutani, K. K. and Anand, K. K. (2001). Adaptogenic activity of a novel, withanolide-free aqueous fraction from the roots of Withania somnifera Dun. Phytother. Res. 15, 311-318.
18. Singh, N., Nath, R., Mishra, N. and Kholi, R. P. (1978). An experimental evaluation of antistress effects of Geriforte (An ayurvedic drug). Quart J. Crud. Drug Res. 3, 125-33.
19. Sreemantulal, S., Nammi, S., Kolanukondal, R., Koppula1, S. and Boini, K. M. (2005). Adaptogenic and nootropic activities of aqueous extract of Vitis vinifera (grape seed): an experimental study in rat model. BMC Compl. Alt. Med. 5, 1472-6882.
20. The Wealth of India, Vol 3, Revised Edition, Publication and Information Directorate, New Delhi: CSIR; 1992.
21. Vagh, V. T., Kapadia, H. D. and Pavri, D. N. (1975). Clinical evaluation of an indigenous geriatric tonic. Probe. 4, 292.